Supporting Information

Enzymatic Lactone-Carbene C–H Insertion to Build Contiguous Chiral Centers

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This PDF file includes:

I. General Procedures

General. Unless otherwise noted, all chemicals and reagents were obtained from commercial suppliers (Sigma-Aldrich, VWR, Alfa Aesar) and used without further purification. Silica gel chromatography was carried out using AMD Silica Gel 60, 230–400 mesh. ^{1}H and ^{13}C NMR spectra were taken using a Bruker Prodigy 400 MHz instrument and are internally referenced to the residual solvent peak (chloroform). Data for ^{1}H NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, ddd = doublet of doublet of doublets), coupling constant (Hz), integration. Sonication was performed using a Qsonica Q500 sonicator. High-resolution mass spectra were obtained at the California Institute of Technology Mass Spectral Facility. Synthetic reactions were monitored using thin layer chromatography (Merck 60 gel plates) using a UV-lamp for visualization.

Chromatography. Analytical reversed-phase high-performance liquid chromatography (HPLC) was carried out using an Agilent 1200 series instrument and a Kromasil C18 column (4.6 × 50 mm, 5 μm) with water and acetonitrile as the mobile phase and visualization at 230 nm for library screening. Analytical normal-phase HPLC was carried out using an Agilent 1200 series instrument and chiral columns Chiralpak IC/IA/IB/OJ-H/OD-H (4.6 mm × 25 cm) with *n*-hexane and isopropanol as the mobile phase and visualization at 230 or 254 nm for chiral separation.

Cloning and site-saturation mutagenesis. Vector pET22b(+) containing a C-terminal 6x-His tag was used for cloning and expression of all enzymes described in this study. Site-saturation mutagenesis was performed using a modified QuikChange™ mutagenesis protocol.[1] Primer sequences are available upon request. The PCR products were digested with *Dpn*I, gel purified, and fragments were assembled using Gibson Mix.[2] The Gibson assembly products were used to directly transform *Escherichia coli* strain BL21 *E. cloni*® (Lucigen). Cells were grown using Luria-Bertani medium (LB) or Hyperbroth (AthenaES) (HB) with 0.1 mg/mL ampicillin (LB_{amp} or HB_{amp}). Electrocompetent *E. coli* cells were prepared following the protocol of Sambrook *et al*.[3] T5 exonuclease, Phusion polymerase, and Taq ligase were purchased from New England Biolabs (NEB, Ipswich, MA). M9-N minimal medium (abbreviated as M9-N buffer; pH 7.4) was used as a buffering system for whole cells, lysates, and purified proteins, unless otherwise specified. M9-N buffer was used without a carbon source; it contains 47.7 mM Na_2HPO_4, 22.0 mM KH_2PO_4, 8.6 mM NaCl, 2.0 mM $MgSO_4$, and 0.1 mM $CaCl_2$.

Determination of hemeprotein concentration.

1. **Preparation of cell lysate**: Aliquots of ~3 mL OD_{600} = 60 cells were prepared in 15-mL conical tubes, which were then placed on wet ice. Cells were lysed by sonication following the program below: sonication for 4 min, 1 second on - 1 second off, 35% amplitude. The sonicated samples were then transferred to two Eppendorf tubes, and then centrifuged down (14,000 rpm, 15 min, 4 ºC). The supernatants (~2.5 mL) were then collected to a 5-mL glass vial for analysis.

2. **Hemechrome assay for protein concentration measurement**: A solution of

NaOH/pyridine was prepared by mixing 1 mL of NaOH aqueous solution (1 M), 2 mL of water and 2 mL of pyridine. To 4.5 mL of NaOH/pyridine solution, 22.5 μL of $K_3Fe(CN)_6$ aqueous solution (0.1 M) were added to make **solution 1**. A **background solution** was prepared by mixing 500 μL M9-N and 500 μL of the NaOH/pyridine solution, which was used for UV background subtraction. When measuring samples with a UV spectrometer, a spectrum of a mixed solution (oxidized spectrum) with 500 μL cell lysate + 500 μL **solution 1** was taken at the wavelength range 380 nm to 650 nm. Subsequently, 5 μL of dithionite solution (0.5 M in 0.1 M NaOH solution) were added to the same sample and mixed by pipetting; a spectrum of this solution (reduced spectrum) was taken at 380 nm to 650 nm. The protein concentration was calculated using the extinction coefficient and dilution factor (2X dilution in volume): $\varepsilon\_[557_{reduced} - 540_{oxidized}] = 23.98\ mM^{-1}cm^{-1}$.[4]

Expression of P411 proteins. *E. coli* BL21 *E. cloni*® cells carrying a plasmid encoding a P411 variant were grown overnight in 5 mL LB_{amp} (37 ºC, 220 rpm). The pre-culture was used to inoculate 45 mL of HB_{amp} in a 125-mL Erlenmeyer flask; this culture was incubated at 37 ºC, 220 rpm for 2 h and 15 min. Cultures were then cooled on ice (40 min), and expression was induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and 1.0 mM 5-aminolevulinic acid (final concentrations). Expression was conducted at room temperature (24 or 22 ºC), at 140 (or 150) rpm, for 20 h (± 20 min). Cultures were then centrifuged (4,500 × g, 5 min, 4 ºC), and the pellets were resuspended to an OD_{600} of 60 in M9-N buffer. Aliquots of the cell suspension (3 mL) were used to determine protein concentration after lysis by sonication. The expression level in OD_{600} = 60 lysates is typically in the range of 3–13 μM for the P411-**C10** variants.

Biotransformations. All the biocatalytic reactions were set up in an anaerobic chamber (oxygen level: < 40 ppm). Resuspended cells (340 μL, diluted to a given OD_{600} with M9-N minimal buffer) were added to 2 mL vials, followed by D-glucose (40 μL, 250 mM in M9-N), aniline derivatives (10 μL of an EtOH stock, 400 or 480 mM), and α-diazo-γ-lactone (**LAD**, 10 μL of an EtOH stock, 400 or 480 mM). Final concentrations were typically 10.0 or 12.0 mM aniline derivative, 10.0 or 12.0 mM **LAD**, and 25 mM D-glucose; final reaction volume was 400 μL. The vials were sealed, shaken inside the anaerobic chamber at room temperature for a set time (600 rpm). After the reaction was completed and the vials removed from the anaerobic chamber, internal standard 1,3,5-trimethoxybenzene (1,3,5-TMOB), *p*-methyl anisole (pMe-anisole), ethyl 2-phenylacetate (PhEA), or allyl phenyl ether (AllylOPh) (20 μL of 20 mM stock solution in acetonitrile) was added followed by acetonitrile (0.58 mL). The mixture was transferred to a 1.7-mL Eppendorf tube, and then subjected to vortexing (15 s × 3) and centrifugation (14,000 rpm, 5 min, 4 °C). A sample of the supernatant (0.8 mL) was transferred to a vial for reverse-phase HPLC analysis.

Reaction screening in 96-well plate format. Libraries (single site-saturation libraries generated employing the "22c-trick" method[1] or collections of heme protein variants) were screened in 96-well plates.

E. coli libraries for P411 variants were cultured in LB_{amp} (350 μL/well) at 37 ºC, 250 rpm and 80% relative humidity overnight. HB_{amp} (950 μL/well) was inoculated with the pre-culture (50 μL/well) and incubated at 37 ºC, 230 rpm, 80% humidity for 2 h and 45

min. The plates were cooled on ice for 30 minutes, and expression was induced with 0.5 mM IPTG and 1.0 mM 5-aminolevulinic acid (final concentrations). Expression was conducted at 22 ºC and 220 rpm for 20 h.

The cells were pelleted (4,500 × g, 5 min, 4 ºC) and resuspended with M9-N buffer (340 µL/well) and D-glucose solution (40 µL/well, in M9-N). The 96-well plate was then transferred to an anaerobic chamber. In the anaerobic chamber, aniline derivative (10 µL/well, 400 mM in EtOH) and **LAD** (10 µL/well, 400 mM in EtOH) were added. The plate was sealed with aluminum foil and shaken inside the anaerobic chamber (600 rpm).

After 24 h, the plate was moved out of the anaerobic chamber. The seal was removed and acetonitrile (580 µL/well) and internal standard (1,3,5-trimethoxybenzene, *p*-methyl anisole, ethyl 2-phenylacetate or allyl phenyl ether; 20 mM in acetonitrile; 20 µL/well) were added. The plate was tightly sealed with a reusable silicone mat, vortexed (15 s × 3) and centrifuged (4,500 × g, 5 min). The supernatant (200 µL/well) was filtered through an AcroPrep 96-well filter plate (0.2 µm) into a shallow-well plate for reversed-phase HPLC analysis (C18 Kromasil column, MeCN:H_2O 50:50, 1.2 mL/min flow, 230 or 254 nm).

II. Directed Evolution of P411-C10 for Lactone-Carbene C–H Insertion

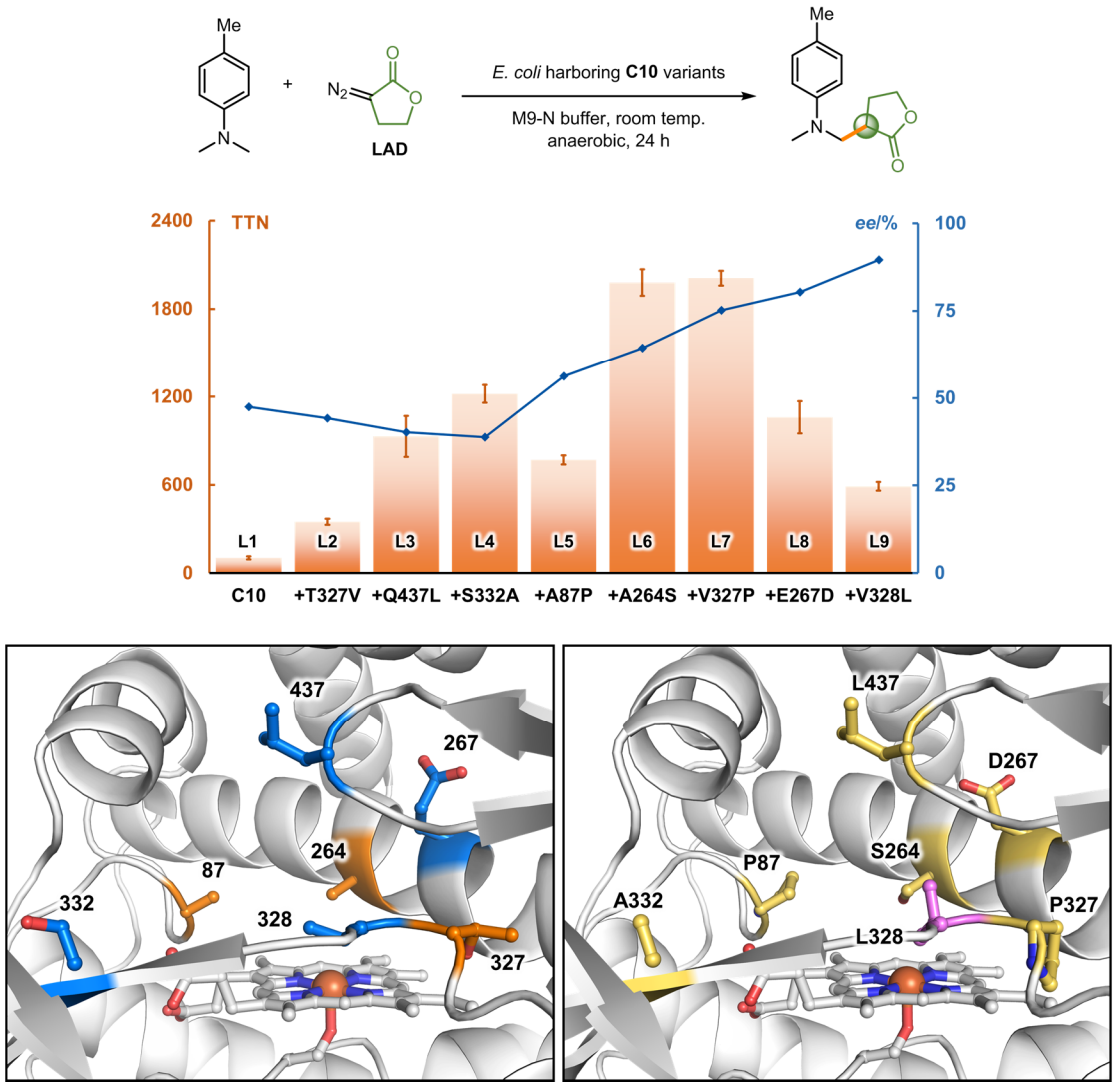


Figure S1. Directed evolution of P411-C10 for lactone-carbene C–H insertion. Reaction conditions: 10 mM 4,*N*,*N*-trimethyl aniline (**1a**), 10 mM α-diazo-γ-lactone (**LAD**), *E. coli* harboring P411-**C10** variants (OD_{600} = 60 for **L1**, **L5** and **L10**, OD_{600} = 30 for the rest), D-glucose (25 mM), M9-N buffer/EtOH (19:1), anaerobic, 24 h. Product formation was quantified by HPLC and TTNs were determined based on protein concentration. The active-site structure in the heme domain of P411-**E10** variant, an enzyme previously evolved for C–H amination with significant homology to P411-**C10**, (pdb: 5UCW) was used to guide site-saturation mutagenesis (left). Mutations obtained from evolution are shown in the active-site structure (right) for comparison (Note: the mutations are generated by Pymol).

Table S1. Detailed information of the evolutionary lineage.

P411-C10 variant	**TTN**	***ee***
C10 (**L1**)	105 ± 10	47%
C10-T327V (**L2**)	350 ± 20	44%
C10-T327V Q437L (**L3**)	930 ± 140	40%
C10-T327V Q437L S332A (**L4**)	1220 ± 60	39%
C10-T327V Q437L S332A A87P (**L5**)	770 ± 30	56%
C10-T327V Q437L S332A A87P A264S (**L6**)	1980 ± 90	64.5%
C10-T327P Q437L S332A A87P A264S (**L7**)	2010 ± 50	75%
C10-T327P Q437L S332A A87P A264S E267D (**L8**)	1060 ± 110	80.5%
C10-T327P Q437L S332A A87P A264S E267D V328L (**L9**)	590 ± 30	90%
C10-T327P Q437L S332A A87P A264S E267D V328R (**L10**)	180 ± 15	–66%

Table S2. Further information on directed evolution experiments.

Round #	**Parent**	**Sites targeted for site-saturation mutagenesis**	**Screen for activity or enantioselectivity**	**Beneficial mutations obtained**
1	**C10**	87, 263, 327, 438	activity	T327V (~3-fold improvement) T327I (~2-fold improvement)
2	**L2**	72, 78, 435, 437	activity	Q437L (~3-fold improvement) Q437I (~3-fold improvement) Q437M (~2.5-fold improvement)
3	**L3**	72, 75, 268, 332	activity	S332A (~1.3-fold improvement) S332C (~1.2-fold improvement)
4	**L4**	87, 263	enantioselectivity	A87P (ee increased to 56%, TTN decreased to ~60% of **L3**)
5	**L5**	264, 82	enantioselectivity	A264S (ee increased to 64.5%, 2.5-fold improvement in TTN)
6	**L6**	395, 327	enantioselectivity	V327P (ee increased to 75%, similar activity to **L5**) V327I (ee increased to 71%,

				~60% decrease in TTN) V327S (ee increased to 74%, ~75% decrease in TTN)
7	**L7**	437, 267	enantioselectivity	E267D (ee increased to 80.5%, ~50% decrease in TTN)
8	**L8**	328, 401	enantioselectivity	V328L (ee increased to 90%, ~40% decrease in TTN) V328R (ee flipped to –66%, ~80% decrease in TTN)

Table S3. Optimization of expression and reaction conditions.

Variant	Enzyme expression conditions (**1**) + Reaction conditions (**1**)	Enzyme expression conditions (**2**) + Reaction conditions (**2**)
L6	OD_{600} = 30 2070 TTN, 72% yield, 64.5% ee	OD_{600} = 60 2760 TTN, 82% yield, 63% ee
L7	OD_{600} = 30 1960 TTN, 86% yield, 75% ee	OD_{600} = 30 2920 TTN, >99% yield, 74.5% ee
L9	OD_{600} = 30 610 TTN, 16% yield, 90% ee	OD_{600} = 60 1380 TTN, 61% yield, 90.5% ee
L10	OD_{600} = 60 180 TTN, 6% yield, –66% ee	OD_{600} = 60 360 TTN, 8% yield, –68% ee

Enzyme expression conditions (**1**) for **Table S1**: 22 ºC, at 150 rpm, for 20 h (± 20 min).

Enzyme expression conditions (**2**): 24 ºC, at 140 rpm, for 20 h (± 20 min).

Reaction conditions (**1**) for **Table S1**: 10 mM 4,*N*,*N*-trimethyl aniline (**1a**), 10 mM LAD, *E. coli* harboring P411-**C10** variants (OD_{600} = 60 for **L1**, **L5** and **L10**, OD_{600} = 30 for the rest), D-glucose (25 mM), M9-N buffer/EtOH (19:1), anaerobic, 24 h.

Reaction conditions (**2**): 12 mM 4,*N*,*N*-trimethyl aniline (**1a**), 12 mM LAD, *E. coli* harboring P411-**C10** variants (OD_{600} = 30 for **L7**, OD_{600} = 60 for the rest), D-glucose (25 mM), M9-N buffer/EtOH (19:1), anaerobic, 24 h.

III. Screening of C10 Lineage for Activity on Different Substrates

Table S4. Aniline derivatives

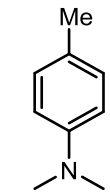
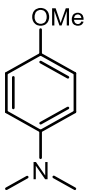
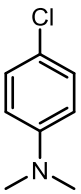
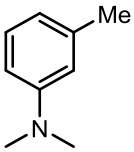
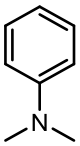
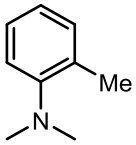
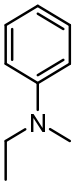
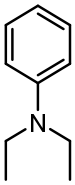
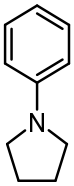
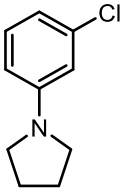
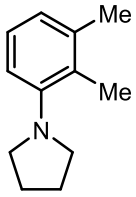
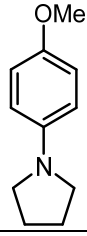
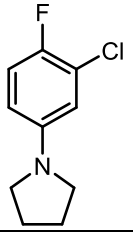
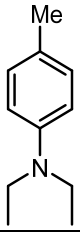
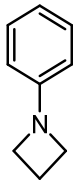
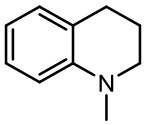
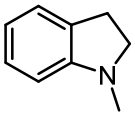
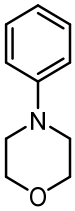
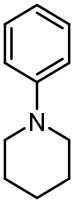
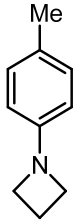
				
S1: pMe-DMA (**1a**)	**S2**: pOMe-DMA (**1b**)	**S3**: pCl-DMA (**1c**)	**S4**: mMe-DMA (**1d**)	**S5**: DMA (**1e**)
				
S6: oMe-DMA (**1f**)	**S7**: Me,Et-Ani (**1g**)	**S8**: DEA (**1h**)	**S9**: Ph-pyr (**1i**)	**S10**: mClPh-pyr (**1j**)
				
S11: diMePh-pyr (**1k**)	**S12**: pOMe-pyr (**1l**)	**S13**: pFmCl-pyr (**1n**)	**S14**: pMe-DEA	**S15**: Ph-aze (**1m**)
				
S16: THQ	**S17**: indoline	**S18**: Ph-mor	**S19**: Ph-pip	**S20**: pTol-aze

Plate screening of different substrates toward lactone-carbene C–H insertion:

Rapid screening (without accurate quantification):

The enzyme lineage **L1** to **L10** was expressed in each line of a 96-well plate following General Procedure in **Section I** (column 2 to column 11 with variants **L1** to **L10**, respectively). Enzymatic reactions were set up with substrate concentration of 10 mM for both LAD and aniline derivative (one substrate in one line). After the reactions were completed, acetonitrile (600 μL/well) was added to reaction plates. The plates were tightly sealed with a reusable silicone mat, vortexed (15 s × 3) and centrifuged (4,500 × g, 5 min). The supernatant (200 μL/well) was filtered through an AcroPrep 96-well filter plate (0.2 μm) into a shallow-well plate for reversed-phase HPLC analysis (C18 Kromasil column, MeCN:H_2O gradient from 40:60 to 100:00, 1.2 mL/min flow, 230 or 254 nm). Promising substrates were then identified with new compound peaks observed on HPLC followed by further confirmation of products with NMR based on reaction scale-up and product

isolation.

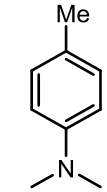
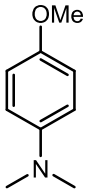
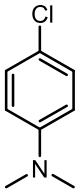
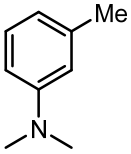
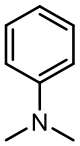
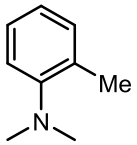
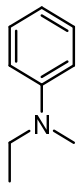
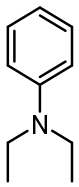
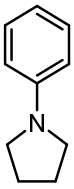
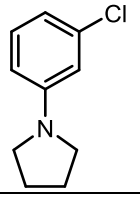
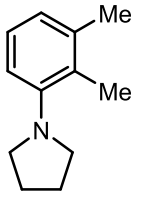
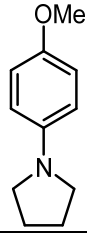
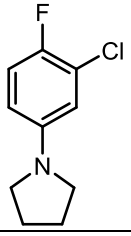
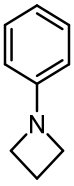
Table S5. Screening result (with promising products: +; without new products: -)

substrate	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
S1 (1a)	+	+	+	+	+	+	+	+	+	+
S2 (1b)	+	+	+	+	+	+	+	+	+	+
S3 (1c)	-	+	+	+	+	+	+	+	+	+
S4 (1d)	-	-	-	+	+	+	+	+	+	+
S5 (1e)	-	-	-	+	+	+	+	+	+	+
S6 (1f)	-	-	-	-	+	+	+	+	+	+
S7 (1g)	-	+	+	+	+	+	+	+	+	+
S8 (1h)	-	-	-	-	+	+	+	+	+	+
S9 (1i)	+	+	+	+	+	+	+	+	+	+
S10 (1j)	-	+	+	+	+	+	+	+	+	+
S11 (1k)	+	+	+	+	+	+	+	+	+	+
S12 (1l)	+	+	+	+	+	+	+	+	+	+
S13	-	-	-	-	+	+	+	+	+	+
S14	-	-	+	+	+	+	+	+	+	+
S15 (1m)	-	-	-	-	-	-	-	-	+	+
S16	-	-	-	+	+	+	+	+	+	-
S17	+	+	+	+	+	+	+	+	+	+
S18	-	-	-	-	-	-	-	-	-	-
S19	-	-	-	-	-	-	-	-	-	-
S20	-	-	-	-	-	-	-	-	-	-

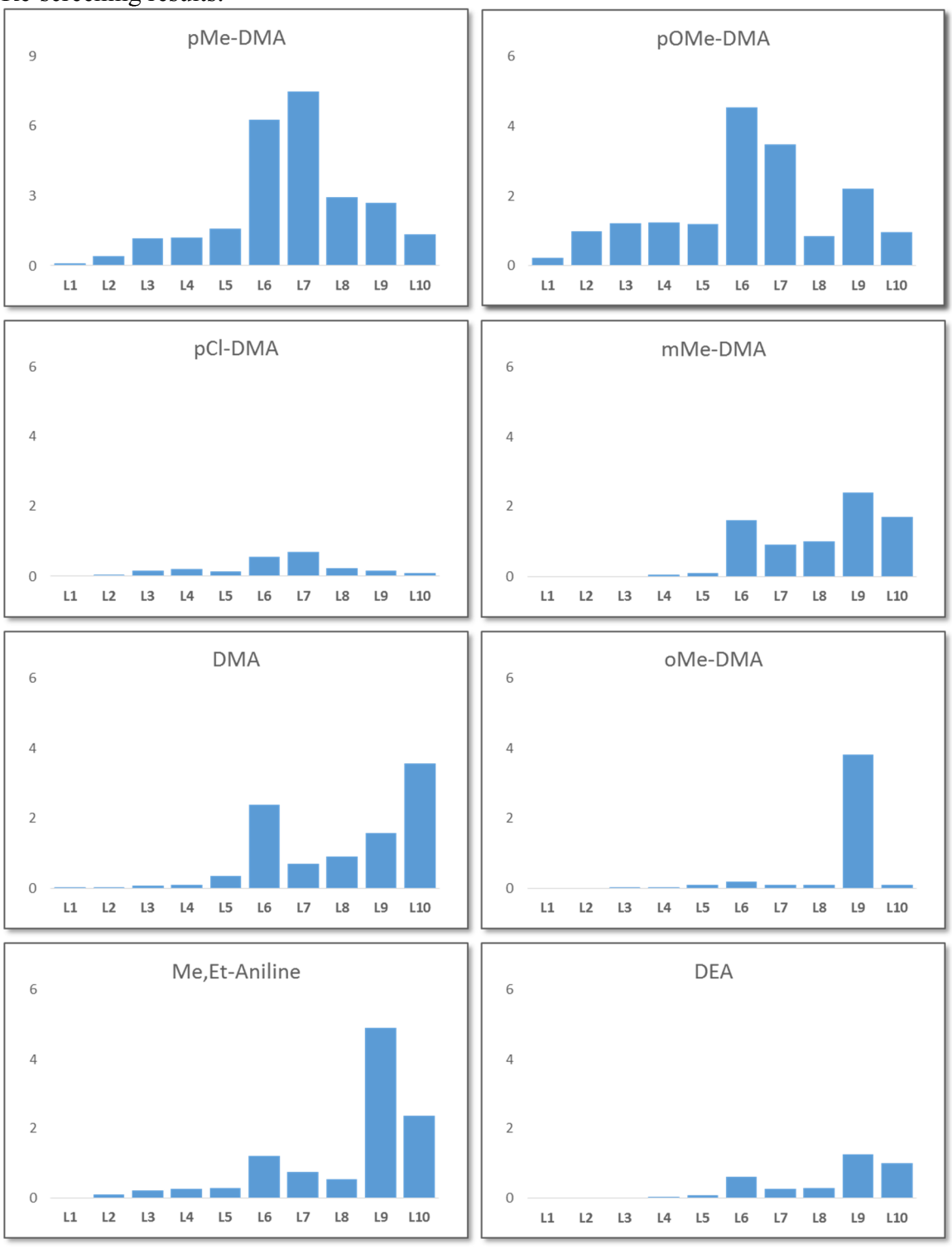
Note: Substrates **S18** to **S20** were found inactive in the lactone-carbene C–H insertion reaction using the enzymes **L1** to **L10**. Substrates **S16** and **S17** were found to generate mixtures of C–H insertion products at multiple sites of the molecules (with poor regio- and stereo-selectivities).

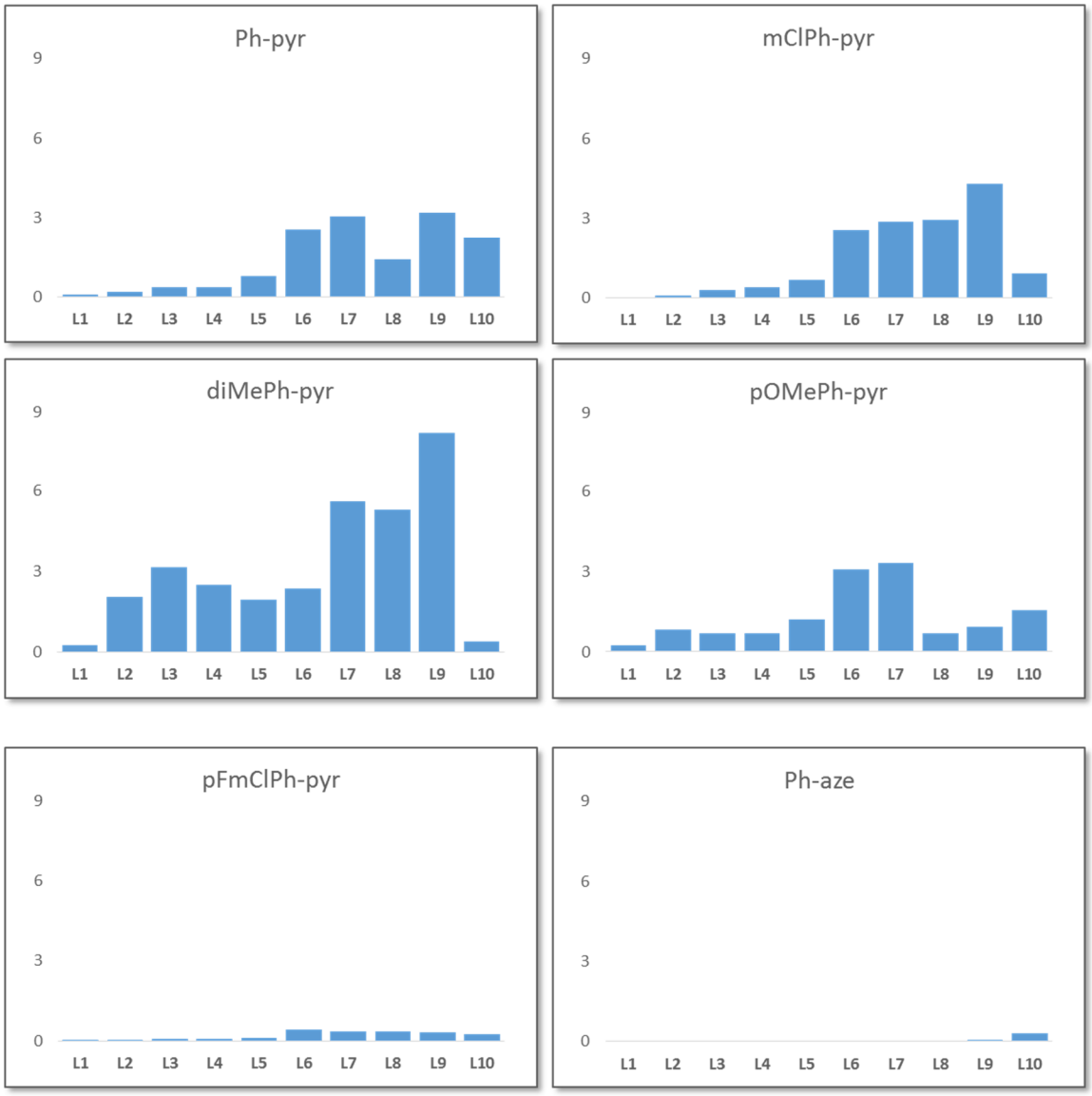
Plate re-screening was performed with the substrates below identified with promising products, following the procedure used for rapid screening but with internal standard for quantification and using specific HPLC methods developed for each substrate (See **Section IV** for the details of product characterization and **Section V** for HPLC calibration curves for product quantification).

Table S6. Active aniline derivatives

				
S1: pMe-DMA (**1a**) **In Std**: pMe-Anisole	**S2**: pOMe-DMA (**1b**) **In Std**: pMe-Anisole	**S3**: pCl-DMA (**1c**) **In Std**: pMe-Anisole	**S4**: mMe-DMA (**1d**) **In Std**: pMe-Anisole	**S5**: DMA (**1e**) **In Std**: PhEA
				
S6: oMe-DMA (**1f**) **In Std**: pMe-Anisole	**S7**: Me,Et-Ani (**1g**) **In Std**: pMe-Anisole	**S8**: DEA (**1h**) **In Std**: PhEA	**S9**: Ph-pyr (**1i**) **In Std**: 1,3,5-TMOB	**S10**: mClPh-pyr (**1j**) **In Std**: pMe-Anisole
				
S11: diMePh-pyr (**1k**) **In Std**: pMe-Anisole	**S12**: pOMe-pyr (**1l**) **In Std**: pMe-Anisole	**S13**: pFmCl-pyr (**1n**) **In Std**: 1,3,5-TMOB	**S15**: Ph-aze (**1m**) **In Std**: AllylOPh	

Re-screening results:





Notes: (1) y axis represents product concentration (mM) in reaction (maximum: 10 mM). (2) The products quantified here correspond to the sum of all the products when different diastereomers (with substrates **S8** to **S15**) or regio-isomers (with substrate **S7**) were formed. (3) Some diastereomers or regio-isomers could be separated from each other (with substrates **S7**, **S8** and **S11** on HPLC), while others did not show separation. (4) Expression level of enzyme variants in plate may be different from that in flasks for validation.

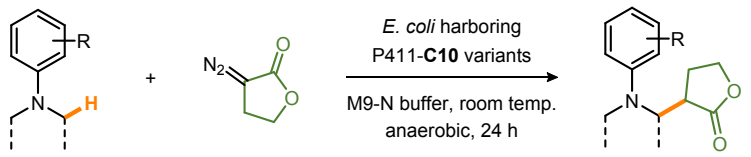
Table S7. Quantification of yield (heat map):

yield 0% 0–0.5% 0.5–2% 2–10% 10–20% 20–50% 50–100%

	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
2a	1.1%	4.2%	11.8%	12.0%	16.0%	62.7%	74.9%	29.6%	27.0%	13.4%
2b	2.2%	10.0%	12.2%	12.3%	11.9%	45.4%	34.9%	8.5%	22.1%	9.7%
2c	0.1%	0.5%	1.5%	2.0%	1.5%	5.5%	7.0%	2.4%	1.7%	0.8%
2d	0.1%	0.1%	0.2%	0.5%	1.0%	16.0%	9.1%	10.1%	23.8%	16.9%
2e	0.1%	0.3%	0.7%	1.0%	3.6%	23.8%	7.0%	9.0%	15.8%	35.5%
2f	0.0%	0.0%	0.0%	0.0%	0.9%	2.0%	1.0%	1.0%	38.1%	1.0%
2g + 2g'	0.0%	1.0%	2.2%	2.5%	2.8%	12.1%	7.4%	5.3%	49.1%	23.7%
2h	0.0%	0.1%	0.2%	0.0%	0.9%	6.2%	2.8%	2.9%	12.5%	10.1%
2i	0.7%	1.7%	3.4%	3.7%	7.6%	25.7%	30.4%	14.0%	32.1%	22.7%
2j	2.6%	20.4%	31.5%	25.1%	19.4%	23.6%	56.2%	53.0%	82.1%	3.8%
2k	0.0%	0.9%	3.0%	3.9%	6.8%	25.6%	29.1%	29.5%	43.2%	9.3%
2l	2.3%	8.3%	6.9%	6.9%	12.1%	30.9%	33.1%	6.9%	9.4%	15.4%
2m	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.4%	2.5%
2n	0.1%	0.2%	0.7%	0.7%	1.0%	4.0%	3.6%	3.4%	3.2%	2.6%

IV. Preparation and Characterization of β-Amino Lactone Products

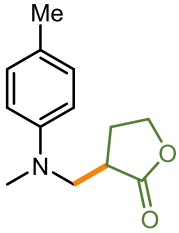
General procedure for enzymatic synthesis of β-amino lactone products:



To 50-mL falcon tubes were added a suspension of *E. coli* expressing P411-**C10** variant (OD_{600} = 60, 30 mL), **LAD** (0.1–0.5 mmol), aniline derivative (1.2 equiv.), D-glucose (~20 mM), M9-N buffer/EtOH (~20:1 v/v) under anaerobic conditions. The tubes were capped and shaken (600 rpm) inside an anaerobic chamber at room temperature for 20–24 h. After the reaction was completed, the reaction mixture was transferred to 500 mL centrifuge bottle, and ~100 mL of hexane/ethyl acetate (1:1) mixed solvent was added. After the bottle was capped, it was shaken vigorously and centrifuged (6,000 × g, 6 min). The organic layer was separated, and the aqueous layer was subjected to three more rounds of extraction. The organic layers were combined, dried over Na_2SO_4 and concentrated under reduced pressure. Purification by silica column chromatography with hexane/ethyl acetate afforded the desired β-amino lactone products.

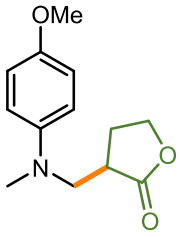
Notes:
1. **C10** variants (usually the most active and/or selective ones) were chosen for each substrate according to the plate screening result in **Section III**. Substrate loading in each reaction was based on rough estimation of the enzymes' activity in plate screening.
2. Total turnovers or yields were not accurately quantified (yields are in the range of 30–80%). The products isolated from these preparative-scale enzymatic reactions were further used for HPLC calibration curves and quantification of analytical-scale reactions in **Section V**.
3. The absolute configuration of the β-amino lactone products was not determined.
4. Preparation of the lactone diazo substrate **LAD** follows the protocol in Chen *et al*.[5]

3-((Methyl(*p*-tolyl)amino)methyl)dihydrofuran-2(3*H*)-one (2a)



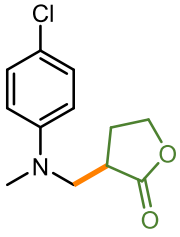
Product **2a** was synthesized using variant **L4**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (d, *J* = 8.2 Hz, 2H), 6.68 (d, *J* = 8.7 Hz, 2H), 4.35 (td, *J* = 8.8, 2.6 Hz, 1H), 4.15 (ddd, *J* = 10.0, 9.1, 6.6 Hz, 1H), 3.94 (dd, *J* = 15.1, 4.5 Hz, 1H), 3.43 (dd, *J* = 15.1, 8.0 Hz, 1H), 2.96 (s, 3H), 2.91 (dddd, *J* = 10.6, 8.6, 8.0, 4.5 Hz, 1H), 2.34 (dddd, *J* = 12.7, 9.0, 6.6, 2.6 Hz, 1H), 2.26 (s, 3H), 2.12 (dtd, *J* = 12.7, 10.2, 8.6 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.48, 146.77, 130.03, 126.65, 113.06, 66.74, 53.31, 39.56, 38.67, 28.10, 20.36. HRMS (TOF) m/z: 220.1311 ($M+H^+$); calc. for [$C_{13}H_{17}NO_2+H^+$]: 220.1332.

3-(((4-Methoxyphenyl)(methyl)amino)methyl)dihydrofuran-2(3*H*)-one (2b)



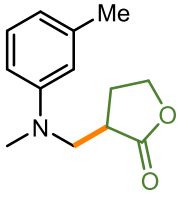
Product **2b** was synthesized using variant **L6**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.85 (d, *J* = 9.1 Hz, 2H), 6.75 (d, *J* = 7.9 Hz, 2H), 4.34 (td, *J* = 8.8, 2.6 Hz, 1H), 4.15 (ddd, *J* = 9.8, 9.1, 6.7 Hz, 1H), 3.86 (dd, *J* = 14.8, 4.3 Hz, 1H), 3.76 (s, 3H), 3.37 (dd, *J* = 14.8, 8.3 Hz, 1H), 2.92 (s, 3H), 2.90–2.82 (m, 1H), 2.34 (dddd, *J* = 11.3, 8.9, 6.7, 2.6 Hz, 1H), 2.18–2.04 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.48, 118.03, 115.22, 115.00, 114.74, 66.74, 55.86, 54.34, 40.16, 38.56, 28.23. HRMS (TOF) m/z: 236.1288 ($M+H^+$); calc. for [$C_{13}H_{17}NO_3+H^+$]: 236.1281.

3-(((4-Chlorophenyl)(methyl)amino)methyl)dihydrofuran-2(3*H*)-one (2c)



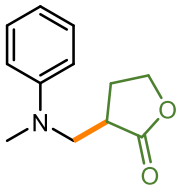
Product **2c** was synthesized using variant **L7**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (d, *J* = 9.1 Hz, 2H), 6.65 (d, *J* = 9.1 Hz, 2H), 4.35 (td, *J* = 8.8, 2.4 Hz, 1H), 4.16 (ddd, *J* = 10.1, 9.1, 6.6 Hz, 1H), 3.93 (dd, *J* = 15.3, 4.7 Hz, 1H), 3.46 (dd, *J* = 15.2, 7.7 Hz, 1H), 2.97 (s, 3H), 2.89 (dddd, *J* = 10.8, 8.8, 7.7, 4.8 Hz, 1H), 2.35 (dddd, *J* = 12.6, 8.8, 6.5, 2.4 Hz, 1H), 2.09 (dddd, *J* = 12.6, 10.8, 10.1, 8.6 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.13, 147.35, 129.25, 122.10, 113.70, 66.67, 53.01, 39.51, 38.65, 27.96. HRMS (TOF) m/z: 240.0785 ($M+H^+$); calc. for [$C_{12}H_{14}ClNO_2+H^+$]: 240.0786.

3-((Methyl(*m*-tolyl)amino)methyl)dihydrofuran-2(3*H*)-one (2d)



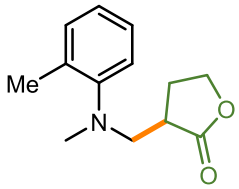
Product **2d** was synthesized using variant **L9**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (dd, *J* = 9.1, 7.4 Hz, 1H), 6.67–6.52 (m, 3H), 4.36 (td, *J* = 8.8, 2.5 Hz, 1H), 4.16 (ddd, *J* = 10.0, 9.1, 6.6 Hz, 1H), 3.98 (dd, *J* = 15.2, 4.6 Hz, 1H), 3.46 (dd, *J* = 15.2, 7.9 Hz, 1H), 2.99 (s, 3H), 2.94 (dddd, *J* = 10.7, 8.8, 8.0, 4.6 Hz, 1H), 2.41–2.33 (m, 1H), 2.32 (s, 3H), 2.13 (dddd, *J* = 12.7, 10.7, 10.0, 8.5 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.40, 148.86, 139.25, 129.35, 118.19, 113.36, 109.84, 66.72, 52.94, 39.42, 38.77, 28.02, 22.07. HRMS (TOF) m/z: 220.1341 ($M+H^+$); calc. for [$C_{13}H_{17}NO_2+H^+$]: 220.1332.

3-((Methyl(phenyl)amino)methyl)dihydrofuran-2(3*H*)-one (2e)



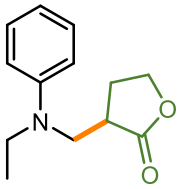
Product **2e** was synthesized using variant **L6**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29–7.22 (m, 3H), 6.75 (dtt, *J* = 7.3, 1.9, 1.0 Hz, 3H), 4.36 (td, *J* = 8.8, 2.6 Hz, 1H), 4.16 (ddd, *J* = 9.9, 9.1, 6.6 Hz, 1H), 3.98 (dd, *J* = 15.2, 4.5 Hz, 1H), 3.48 (dd, *J* = 15.2, 7.8 Hz, 1H), 3.00 (s, 3H), 2.93 (dddd, *J* = 10.8, 8.8, 7.9, 4.6 Hz, 1H), 2.36 (dddd, *J* = 12.6, 8.9, 6.6, 2.5 Hz, 1H), 2.13 (dddd, *J* = 12.7, 10.7, 10.0, 8.5 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.37, 148.79, 129.52, 117.24, 112.61, 66.73, 52.96, 39.38, 38.78, 28.03. HRMS (TOF) m/z: 206.1167 ($M+H^+$); calc. for [$C_{12}H_{15}NO_2+H^+$]: 206.1176.

3-((Methyl(*o*-tolyl)amino)methyl)dihydrofuran-2(3*H*)-one (2f)



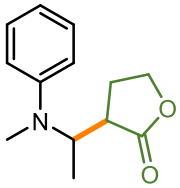
Product **2f** was synthesized using variant **L9**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24–7.17 (m, 2H), 7.14 (d, J = 7.8 Hz, 1H), 7.04 (td, J = 7.3, 1.5 Hz, 1H), 4.35 (td, J = 8.8, 3.0 Hz, 1H), 4.17 (ddd, J = 9.6, 9.1, 6.9 Hz, 1H), 3.62 (dd, J = 12.9, 4.2 Hz, 1H), 3.00 (dd, J = 12.9, 10.3 Hz, 1H), 2.78 (tdd, J = 10.1, 8.8, 4.3 Hz, 1H), 2.69 (s, 3H), 2.33 (s, 3H), 2.32 – 2.24 (m, 1H), 2.12 (dtd, J = 12.9, 9.8, 8.6 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.66, 150.84, 133.89, 131.35, 126.82, 124.10, 120.95, 66.97, 55.95, 43.43, 38.63, 28.13, 18.12. HRMS (TOF) m/z: 220.1332 ($M+H^+$); calc. for [$C_{13}H_{17}NO_2+H^+$]: 220.1332.

3-((Ethyl(pheny)amino)methyl)dihydrofuran-2(3*H*)-one (2g)



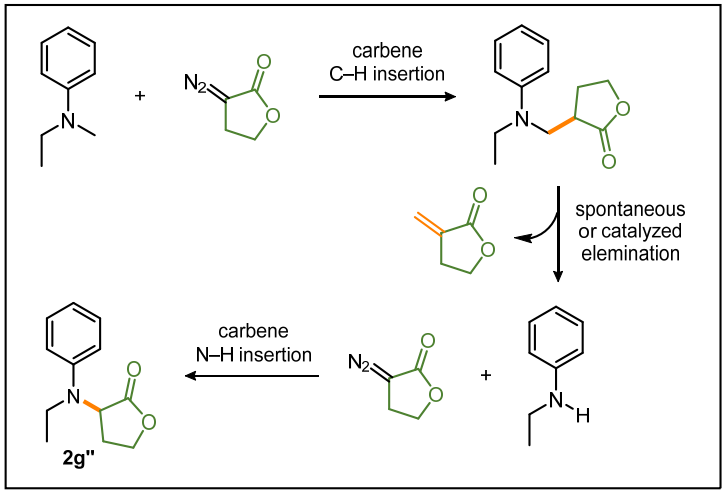
Product **2g** was synthesized using variants **L9** and **L10**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.20 (m, 3H), 6.82–6.64 (m, 3H), 4.36 (td, J = 8.8, 2.5 Hz, 1H), 4.16 (ddd, J = 9.9, 9.1, 6.6 Hz, 1H), 3.91 (dd, J = 15.1, 4.6 Hz, 1H), 3.55–3.30 (m, 3H), 2.93 (tdd, J = 10.8, 8.3, 4.6 Hz, 1H), 2.38 (dddd, J = 12.5, 8.9, 6.6, 2.5 Hz, 1H), 2.13 (dtd, J = 12.6, 10.3, 8.7 Hz, 1H), 1.15 (t, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.47, 147.63, 129.56, 117.01, 112.97, 66.77, 50.65, 46.17, 39.10, 28.00, 12.21. HRMS (TOF) m/z: 220.1311 ($M+H^+$); calc. for [$C_{13}H_{17}NO_2+H^+$]: 220.1332.

3-(1-(Methyl(phenyl)amino)ethyl)dihydrofuran-2(3*H*)-one (2g')

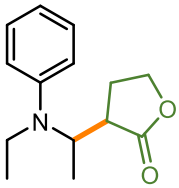


Product **2g'** was observed in the enzymatic reaction using variant **L10** and was obtained in a mixture form with a side product **2g''**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31–7.21 (m, 2H), 6.91–6.70 (m, 3H), 4.57 (dd, J = 8.8, 11.1 Hz, 2H), 4.35 (td, J = 8.7, 3.1 Hz, 1H), 4.14 (td, J = 9.2, 7.1 Hz, 1H), 2.91 (td, J = 9.8, 3.9 Hz, 1H), 2.80 (s, 3H), 2.37–2.18 (m, 2H), 1.31 (d, J = 6.9 Hz, 3H). HRMS (TOF) m/z: 220.1342 ($M+H^+$); calc. for [$C_{13}H_{17}NO_2+H^+$]: 220.1332.

Potential pathway for side product **2g''** (which was also observed sparingly in the enzymatic reactions with variants **L6** and **L9**):

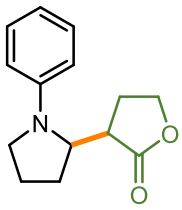


3-(1-(Ethyl(phenyl)amino)ethyl)dihydrofuran-2(3*H*)-one (2h)



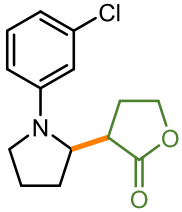
Product **2h** was synthesized with a diastereomeric ratio of 16:1 using variant **L9**. Further silica column purification using dichloromethane/ acetone/hexane as eluent was able to afford the pure major diastereomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30–7.21 (m, 3H), 6.85 (dd, *J* = 9.0, 1.1 Hz, 2H), 6.76 (tt, *J* = 7.3, 1.0 Hz, 1H), 4.51 (qd, *J* = 6.9, 3.7 Hz, 1H), 4.35 (td, *J* = 8.8, 2.6 Hz, 1H), 4.13 (ddd, *J* = 9.8, 8.9, 6.9 Hz, 1H), 3.37–3.18 (m, 2H), 2.93 (ddd, *J* = 10.8, 9.1, 3.7 Hz, 1H), 2.37–2.25 (m, 1H), 2.25–2.15 (m, 1H), 1.31 (d, *J* = 6.9 Hz, 3H), 1.15 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.12, 147.76, 129.57, 117.65, 114.35, 66.51, 52.38, 42.43, 39.62, 24.27, 14.65, 14.55. HRMS (TOF) m/z: 234.1483 ($M+H^+$); calc. for [$C_{14}H_{19}NO_2+H^+$]: 234.1489.

3-(1-Phenylpyrrolidin-2-yl)dihydrofuran-2(3*H*)-one (2i)



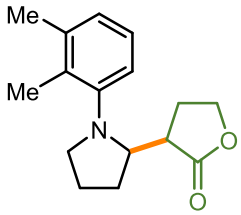
Product **2i** was synthesized with a diastereomeric ratio of 9:1 using variant **L9**. The major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.22 (m, 2H), 6.81–6.62 (m, 3H), 4.47 (dt, *J* = 7.9, 3.7 Hz, 1H), 4.38 (ddd, *J* = 9.0, 6.5, 4.6 Hz, 1H), 4.17–4.08 (m, 1H), 3.57 (ddd, *J* = 9.2, 6.9, 5.6 Hz, 1H), 3.41 (td, *J* = 9.9, 3.9 Hz, 1H), 3.34–3.25 (m, 1H), 2.36–2.19 (m, 1H), 2.18–1.99 (m, 4H), 1.81 (dtd, *J* = 12.7, 6.2, 3.5 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.13, 146.67, 129.55, 116.89, 112.90, 66.73, 56.78, 49.63, 41.16, 27.89, 24.36, 23.91. HRMS (TOF) m/z: 232.1321 ($M+H^+$); calc. for [$C_{14}H_{17}NO_2+H^+$]: 232.1332.

3-(1-(3-Chlorophenyl)pyrrolidin-2-yl)dihydrofuran-2(3*H*)-one (2j)



Product **2j** was synthesized with a diastereomeric ratio of 9:1 using variant **L9**. The major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (t, *J* = 8.1 Hz, 1H), 6.69 (ddd, *J* = 7.8, 1.9, 0.8 Hz, 1H), 6.62 (t, *J* = 2.2 Hz, 1H), 6.54 (ddd, *J* = 8.4, 2.5, 0.8 Hz, 1H), 4.44–4.38 (m, 1H), 4.38–4.25 (m, 1H), 4.13 (td, *J* = 9.0, 8.0 Hz, 1H), 3.50 (ddd, *J* = 9.3, 7.0, 5.7 Hz, 1H), 3.34 (td, *J* = 10.0, 4.0 Hz, 1H), 3.26 (dt, *J* = 9.3, 7.4 Hz, 1H), 2.36–2.18 (m, 1H), 2.16–1.95 (m, 4H), 1.83–1.74 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.81, 147.66, 135.38, 130.42, 116.75, 112.78, 111.00, 66.67, 56.85, 49.65, 41.04, 27.90, 24.25, 23.86. HRMS (TOF) m/z: 266.0937 ($M+H^+$); calc. for [$C_{14}H_{16}ClNO_2+H^+$]: 266.0942.

3-(1-(2,3-Dimethylphenyl)pyrrolidin-2-yl)dihydrofuran-2(3*H*)-one (2k)

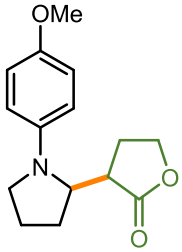


Product **2k** was synthesized with a diastereomeric ratio of 1:1.6 using variant **L9**. The diastereomers can be easily separated by silica chromatography.

Diastereomer-1 (**2k2**, major): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04 (d, *J* = 4.6 Hz, 2H), 6.88 (t, *J* = 4.3 Hz, 1H), 4.14–4.02 (m, 3H), 3.51 – 3.42 (m, 1H), 2.77–2.68 (m, 1H), 2.62 (td, *J* = 9.0, 5.3 Hz, 1H), 2.39–2.27 (m, 1H), 2.26 (s, 3H), 2.23 (s, 3H), 2.19–2.04 (m, 2H), 2.00–1.82 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.08, 149.05, 138.00, 132.78, 125.94, 125.31, 118.85, 66.71, 60.32, 56.02, 44.40, 31.13, 25.56, 24.45, 20.83, 14.59. HRMS (TOF) m/z: 260.1631 ($M+H^+$); calc. for

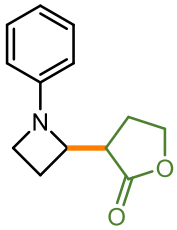
[$C_{16}H_{21}NO_2$+H^+]: 260.1645.
Diastereomer-2 (**2k1**, minor): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12–7.02 (m, 2H), 6.91 (dd, *J* = 6.7, 1.7 Hz, 1H), 4.29 (td, *J* = 9.1, 3.0 Hz, 1H), 4.14 (ddd, *J* = 8.0, 7.3, 4.7 Hz, 1H), 4.08 (td, *J* = 9.3, 7.4 Hz, 1H), 3.45 (ddd, *J* = 9.1, 7.3, 5.5 Hz, 1H), 2.91 (td, *J* = 9.7, 4.6 Hz, 1H), 2.65 (ddd, *J* = 9.1, 7.9, 6.6 Hz, 1H), 2.27 (s, 3H), 2.23–2.05 (m, 5H), 1.99–1.82 (m, 3H), 1.71–1.61 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.88, 146.87, 138.26, 132.69, 126.03, 125.29, 117.37, 67.16, 58.57, 54.78, 40.52, 26.16, 23.61, 23.02, 20.80, 14.36. HRMS (TOF) m/z: 260.1628 (M+H^+); calc. for [$C_{16}H_{21}NO_2$+H^+]: 260.1645.

3-(1-(4-Methoxyphenyl)pyrrolidin-2-yl)dihydrofuran-2(3*H*)-one (2l)



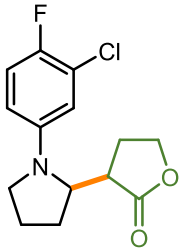
Product **2l** was synthesized using variant **L7** (with a diastereomeric ratio of 1.4:1) and **L9** (with a diastereomeric ratio of 4:1). The major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.86 (d, *J* = 9.1 Hz, 2H), 6.65 (d, *J* = 9.1 Hz, 2H), 4.35 (ddd, *J* = 8.7, 7.1, 4.4 Hz, 2H), 4.17–4.07 (m, 1H), 3.76 (s, 3H), 3.53 (ddd, *J* = 8.9, 6.7, 5.6 Hz, 1H), 3.32 (td, *J* = 9.9, 4.0 Hz, 1H), 3.19 (dt, *J* = 8.8, 7.4 Hz, 1H), 2.33–1.91 (m, 5H), 1.81–1.70 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.33, 151.78, 141.48, 115.21, 114.20, 66.79, 57.28, 55.99, 50.53, 41.28, 27.85, 24.44, 23.82. HRMS (TOF) m/z: 262.1421 (M+H^+); calc. for [$C_{15}H_{19}NO_3$+H^+]: 262.1438.

3-(1-Phenylazetidin-2-yl)dihydrofuran-2(3*H*)-one (2m)



Product **2m** was synthesized with a diastereomeric ratio of >20:1 using variant **L10**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25–7.19 (m, 2H), 6.78 (tt, *J* = 7.4, 1.1 Hz, 1H), 6.57–6.42 (m, 2H), 4.63 (ddd, *J* = 8.3, 7.0, 5.0 Hz, 1H), 4.45 (td, *J* = 9.0, 3.0 Hz, 1H), 4.29 (td, *J* = 9.3, 7.2 Hz, 1H), 3.95 (ddd, *J* = 8.9, 7.1, 4.1 Hz, 1H), 3.67 (dt, *J* = 8.8, 7.3 Hz, 1H), 3.30 (td, *J* = 9.7, 5.1 Hz, 1H), 2.63–2.51 (m, 1H), 2.43–2.31 (m, 2H), 2.21 (ddt, *J* = 11.4, 8.8, 7.2 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.93, 151.30, 129.28, 118.40, 111.82, 67.28, 62.19, 50.18, 43.12, 23.63, 18.92. HRMS (TOF) m/z: 218.1183 (M+H^+); calc. for [$C_{13}H_{15}NO_2$+H^+]: 218.1176.

3-(1-(3-Chloro-4-fluorophenyl)pyrrolidin-2-yl)dihydrofuran-2(3*H*)-one (2n)



Product **2n** was synthesized with a diastereomeric ratio of 13:1 using variant **L9**. The major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.01 (t, *J* = 8.9 Hz, 1H), 6.63 (dd, *J* = 6.1, 3.0 Hz, 1H), 6.48 (dt, *J* = 9.1, 3.4 Hz, 1H), 4.40–4.31 (m, 2H), 4.14 (td, *J* = 8.9, 7.9 Hz, 1H), 3.49 (ddd, *J* = 9.1, 7.1, 5.5 Hz, 1H), 3.28 (td, *J* = 9.9, 4.0 Hz, 1H), 3.21 (dt, *J* = 9.1, 7.4 Hz, 1H), 2.30–2.18 (m, 1H), 2.13–1.97 (m, 4H), 1.83–1.73 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.80, 150.72 (d, *J* = 239.4 Hz), 143.81 (d, *J* = 2.0 Hz), 121.48 (d, *J* = 18.4 Hz), 117.05 (d, *J* = 21.8 Hz), 114.02, 111.82 (d, *J* = 6.1 Hz), 66.69, 57.22, 50.19, 41.06, 27.98, 24.36, 23.81. HRMS (TOF) m/z: 284.0839 (M+H^+); calc. for [$C_{14}H_{15}FClNO_2$+H^+]: 284.0848.

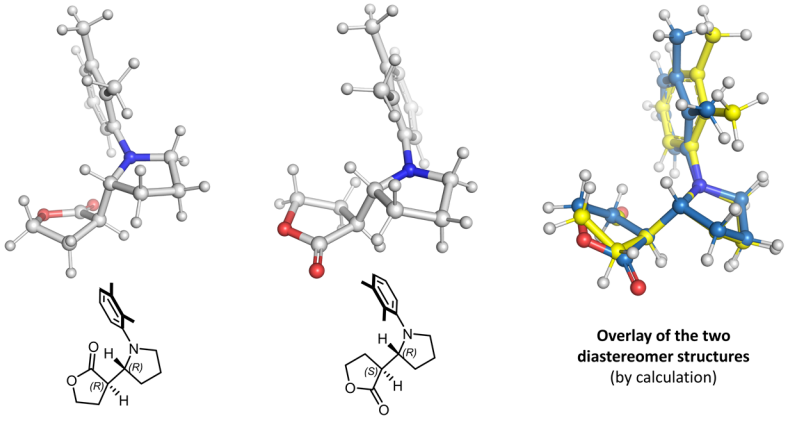
Assignment of diastereomers **2k1** and **2k2** based on computational calculation. (Note: Attempts to get the crystal structures of products **2k1** and **2k2** were not fruitful; therefore, we turned to computational NMR prediction,[6] which provides helpful information for relative configuration assignment of the diastereomers but does not allow a definitive conclusion. Further evidence needs to be included before making final conclusions.)

Comparison of experimental and calculated ^{1}H NMR chemical shifts:

Experimental		Calculated	
2k2 (major)	**2k1** (minor)	(*R*,*S*)-configuration	(*R*,*R*) -configuration
^{1}H NMR (ppm)	^{1}H NMR (ppm)	^{1}H NMR (ppm)	^{1}H NMR (ppm)
7.04	7.06	6.94	6.97
7.04	7.06	6.92	6.87
6.88	6.91	6.72	6.76
4.08	4.29	4.34	4.38
4.08	**4.14**	**4.08**	**4.24**
4.08	4.08	3.97	3.99
3.47	3.45	3.72	3.66
2.72	**2.91**	**2.66**	**3.05**
2.62	**2.65**	**2.61**	**2.67**
2.32	2.27	2.44	2.39
2.26	2.27	2.40	2.24
2.26	2.27	2.38	2.24
2.26	2.16	2.24	2.15
2.23	2.16	2.03	1.97
2.23	2.16	1.98	1.97
2.23	2.16	1.96	1.97
2.12	2.16	1.96	1.97
2.12	**1.93**	1.94	**1.87**
1.92	**1.93**	**1.89**	**1.82**
1.92	**1.93**	**1.81**	**1.82**
1.92	**1.66**	**1.79**	**1.69**

Structural optimization by DFT (density functional theory) calculation suggests that the aromatic rings of the two diastereomers possess slightly different orientations in their most favorable conformations. This difference is expected to cause different deshielding effects on the protons on the pyrrolidine ring and also at the α-position of the lactone structure, which will further lead to different chemical shifts. The computational results show good consistence with the experimental data.

proton assignment	Experimental		Calculated	
	2k2 (major)	**2k1** (minor)	(*R*,*S*)-configuration	(*R*,*R*) -configuration
α-N-C**H**	**4.08**	**4.14**	**4.08**	**4.24**
α-N-C**H**$_2^A$	**3.47**	**3.45**	**3.72**	**3.66**
α-N-C**H**$_2^B$	**2.72**	**2.65**	**2.66**	**2.67**
α-CO-C**H**	**2.62**	**2.91**	**2.61**	**3.05**



Through the comparison of ^{1}H NMR chemical shifts by DFT calculation with the experimental data, **2k2** likely corresponds to (*R*,*S*)-configuration, and **2k1** is probably with (*R*,*R*)-configuration (relative configurations here). *DFT calculation for NMR prediction using B3LYP/6-31G(d), for structure optimization using B3LYP/3-21G.

For the products **2h** to **2j**, **2l** and **2n**, the major diastereomers made using variant **L9** share the same relative configuration with the minor diastereomer **2k1** made from substrate **1k** using variants **L7** to **L9** according to NMR analysis (experimentally). The steric effect of dimethyl substitutions on the phenyl ring may be the major factor leading to the different diastereoselectivities.

V. Analysis of Enzymatic Lactone-Carbene C–H Insertion

All enzymatic reactions for lactone-carbene C–H insertion at analytical scale were conducted following the general procedure described below and analyzed with HPLC. All TTNs for different products were determined using the HPLC standard curves of the corresponding products obtained from the preparative-scale enzymatic reactions in **Section IV**.

General procedure for analytical-scale reactions:
To a 2 mL vial were added degassed suspension of *E. coli* expressing the P411-**C10** variant (under expression conditions (**2**) in **Section II**) in M9-N buffer (OD_{600} = 30 or 60, 340 μL), aniline derivatives (10 μL of 480 mM stock solution in EtOH, 12 mM), **LAD** (10 μL of 480 stock solution in EtOH, 12 mM), D-glucose (40 μL of 250 mM stock solution in M9-N buffer, 25 mM) under anaerobic conditions. The vial was capped and shaken at 600 rpm at room temperature for 24 h. Reactions for every substrate were set up in triplicate or quadruplicate. After the reactions were completed, internal standard (20 μL of 20 mM stock solution in acetonitrile, following **Table S6**) was added to the reaction vials followed by acetonitrile (0.58 mL). The mixture was transferred to a 1.7-mL microcentrifuge tube, and then vortexed (15 seconds × 3) and centrifuged (14,000 rpm, 5 min). For HPLC analysis, 0.8 mL of supernatant were taken. TTN was calculated based on measured protein concentration.

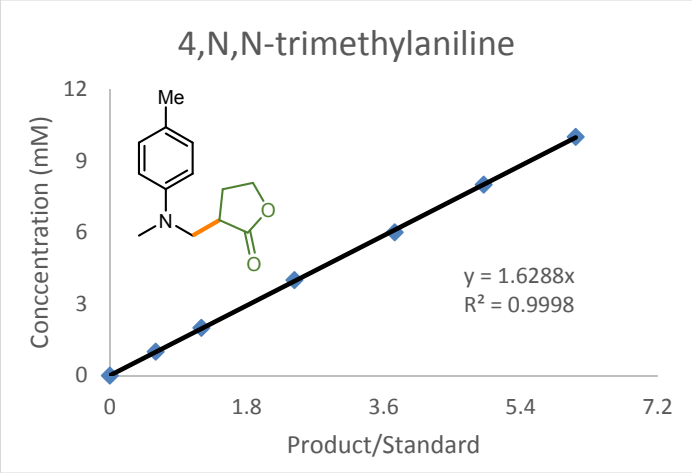
Another set of enzymatic reactions was set up following the same procedure. After the reactions were completed, extraction of products with 0.6 mL of hexane/ethyl acetate (1:1) followed by vortexing and centrifugation afforded non-aqueous organic solutions of the desired products. Enantiomeric excess of the enzymatic reactions was measured using these organic solutions by normal-phase chiral HPLC.

Note: Diastereomeric ratios (d.r.) of the products (if applicable) were determined by NMR and/or HPLC at wavelength of 254 nm (or 230 nm). The two methods show good consistency for dr determination within difference of ±2%.

HPLC standard curve:
All data points represent the average of duplicate runs. The calibration curves depict product concentration in mM (y-axis) against the ratio of product area to internal standard area on the HPLC (x-axis).

3-((Methyl(*p*-tolyl)amino)methyl)dihydrofuran-2(3*H*)-one (2a)

HPLC calibration curve (*p*Me-anisole as internal standard):



Analysis Data:

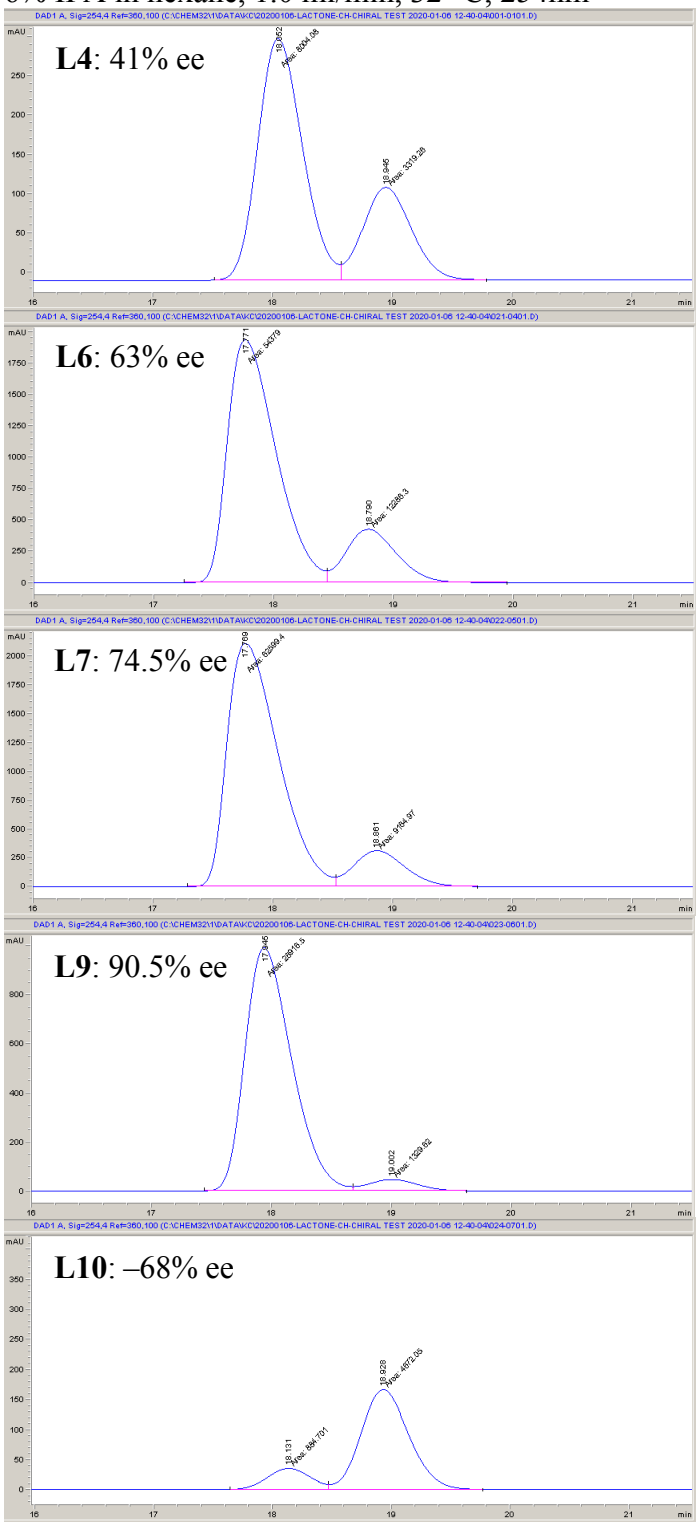
L6 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2a-L6_a	6946.0	1177.9	5.8969	9.6049	3.57	2694	
2a-L6_b	7124.5	1181.0	6.0326	9.8259	3.57	2756	
2a-L6_c	7387.5	1191.3	6.2012	10.1005	3.57	2833	2761

L7 OD_{600} = 30	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2a-L7_a	8503.9	1178.9	7.2134	11.7492	4.10	2865	
2a-L7_b	8814.0	1180.6	7.4657	12.1601	4.10	2965	
2a-L7_c	8693.1	1201.0	7.2382	11.7896	4.10	2875	
2a-L7_d	8801.1	1170.2	7.5210	12.2502	4.10	2987	2923

L9 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2a-L9_a	5770.5	1207.4	4.7793	7.7845	5.18	1502	
2a-L9_b	5860.1	1146.8	5.1100	8.3231	5.18	1606	
2a-L9_c	5807.0	1173.3	4.9493	8.0614	5.18	1556	1555

L10 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2a-L10_a	757.5	1282.2	0.5908	0.9623	2.71	355	
2a-L10_b	781.0	1303.6	0.5991	0.9758	2.71	360	
2a-L10_c	767.1	1260.3	0.6087	0.9914	2.71	366	361

Chiral HPLC trace:
Chiralpak OD-H, 6% IPA in hexane, 1.0 ml/min, 32 °C, 254nm

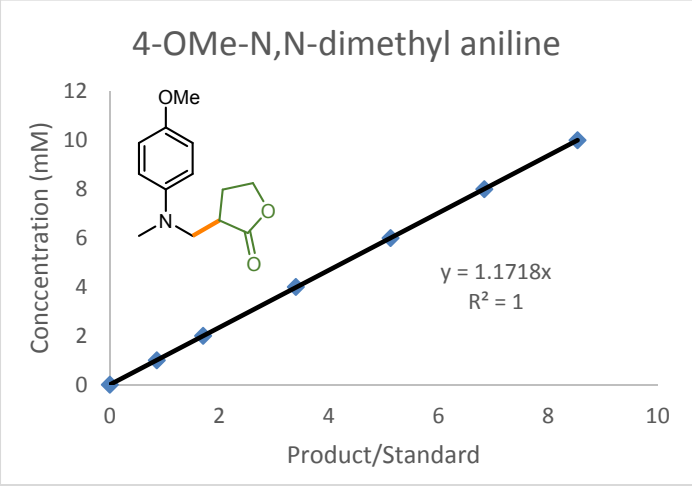


Area% report for enzymatically produced **2a**:

2a by variant **L4**			**2a** by variant **L6**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
18.05	8004.1	70.69	17.77	54379	81.57
18.95	3319.3	29.31	18.79	12286.3	18.43
Total	11323.4	100.00	Total	66665.3	100.00
2a by variant **L7**			**2a** by variant **L9**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
17.77	62599.4	87.23	17.95	26916.5	95.29
18.86	9165.0	12.77	19.00	1329.6	4.71
Total	71764.4	100.00	Total	28246.1	100.00
2a by variant **L10**					
Retention Time (min)	Area (mAU*s)	Area %			
18.13	884.7	15.92			
18.93	4672.1	84.08			
Total	5556.8	100.00			

3-(((4-Methoxyphenyl)(methyl)amino)methyl)dihydrofuran-2(3*H*)-one (2b)

HPLC calibration curve (*p*Me-anisole as internal standard):



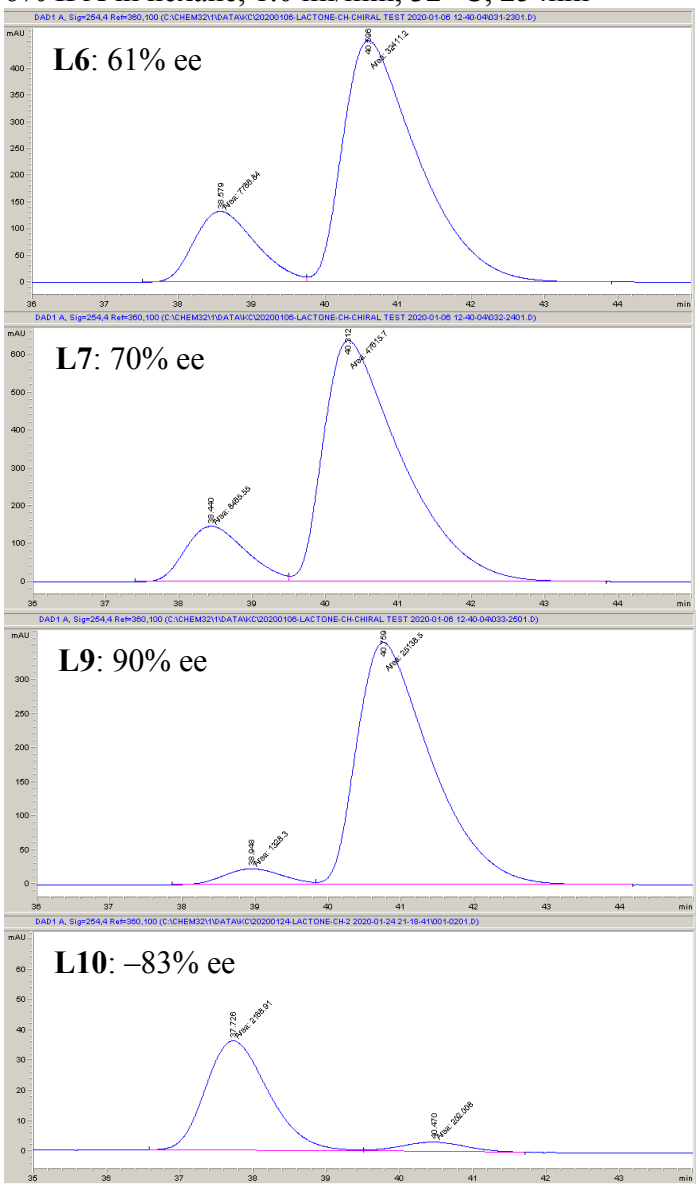
Analysis Data:

L6 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/µM	TTN	Avg. TTN
2b-L6_a	7269.6	1175.4	6.1848	7.2473	3.57	2032	
2b-L6_b	7306.7	1168.5	6.2531	7.3273	3.57	2055	
2b-L6_c	7300.7	1173.5	6.2213	7.2901	3.57	2044	2044

L7 OD_{600} = 30	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2b-L7_a	8352.8	1184.0	7.0547	8.2667	4.40	1878	
2b-L7_b	8083.9	1180.5	6.8479	8.0243	4.40	1823	
2b-L7_c	8109.2	1200.5	6.7549	7.9153	4.40	1798	1833

L9 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2b-L9_a	5711.2	1165.9	4.8985	5.7401	5.18	1108	
2b-L9_b	6011.9	1184.9	5.0738	5.9454	5.18	1147	
2b-L9_c	6498.1	1203.1	5.4011	6.3290	5.18	1221	1159

Chiral HPLC trace:
Chiralpak OD-H, 6% IPA in hexane, 1.0 ml/min, 32 °C, 254nm

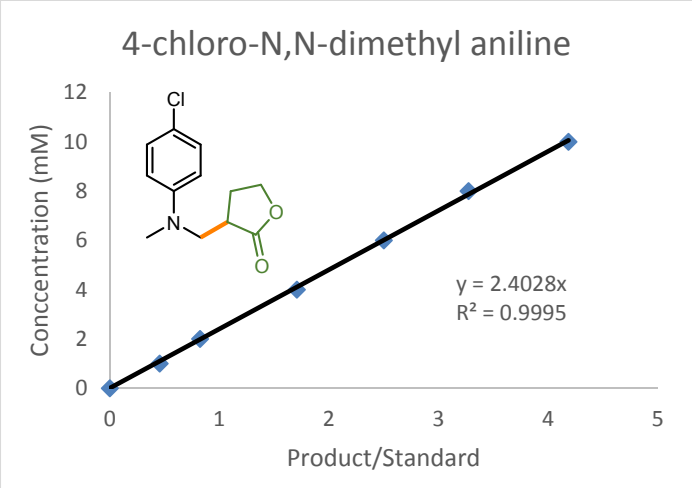


Area% report for enzymatically produced **2b**:

2b by variant **L6**			**2b** by variant **L7**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
38.58	7786.8	19.37	38.44	8465.6	15.10
40.60	32411.2	80.63	40.31	47615.7	84.90
Total	40198.0	100.00	Total	56081.3	100.00
2b by variant **L9**			**2b** by variant **L10**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
38.95	1328.3	5.02	37.73	2168.9	91.48
40.76	25138.5	94.98	40.47	202.0	8.52
Total	26466.8	100.00	Total	2370.9	100.00

3-(((4-Chlorophenyl)(methyl)amino)methyl)dihydrofuran-2(3*H*)-one (2c)

HPLC calibration curve (1,3,5-TMOB as internal standard):

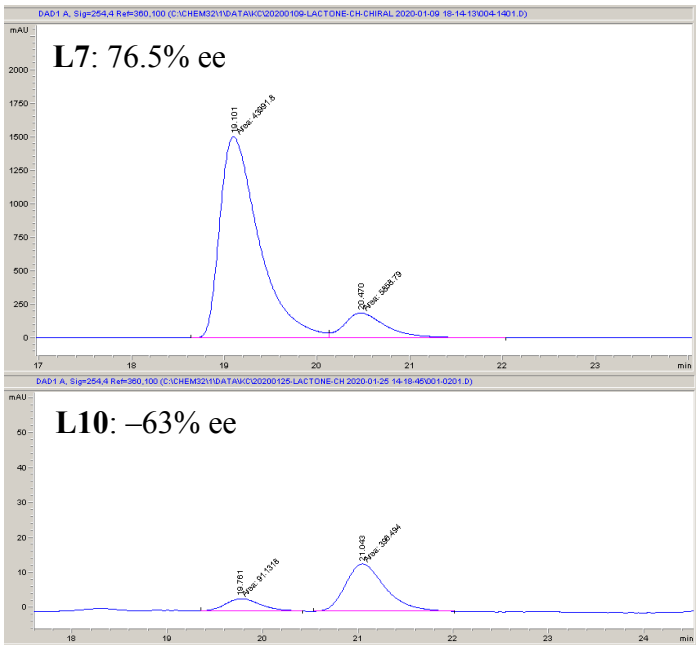


Analysis Data:

L7 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/µM	TTN	Avg. TTN
2c-L7_a	2074.3	1354.6	1.5313	3.6794	8.80	418	
2c-L7_b	2092.0	1363.4	1.5344	3.6869	8.80	419	
2c-L7_c	2131.2	1366.2	1.5599	3.7482	8.80	426	421

Chiral HPLC trace:

Chiralpak IA, 3% IPA in hexane, 1.2 ml/min, 32 °C, 254nm

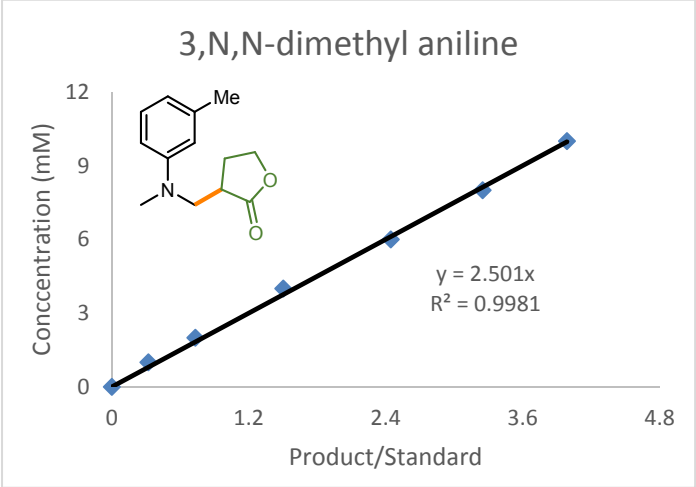


Area% report for enzymatically produced **2c**:

2c by variant **L7**			**2c** by variant **L10**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
19.10	43991.8	88.25	19.76	91.1	18.68
20.47	5858.8	11.75	21.04	396.5	81.32
Total	49850.6	100.00	Total	487.6	100.00

3-((Methyl(*m*-tolyl)amino)methyl)dihydrofuran-2(3*H*)-one (2d)

HPLC calibration curve (*p*Me-anisole as internal standard):



Analysis Data:

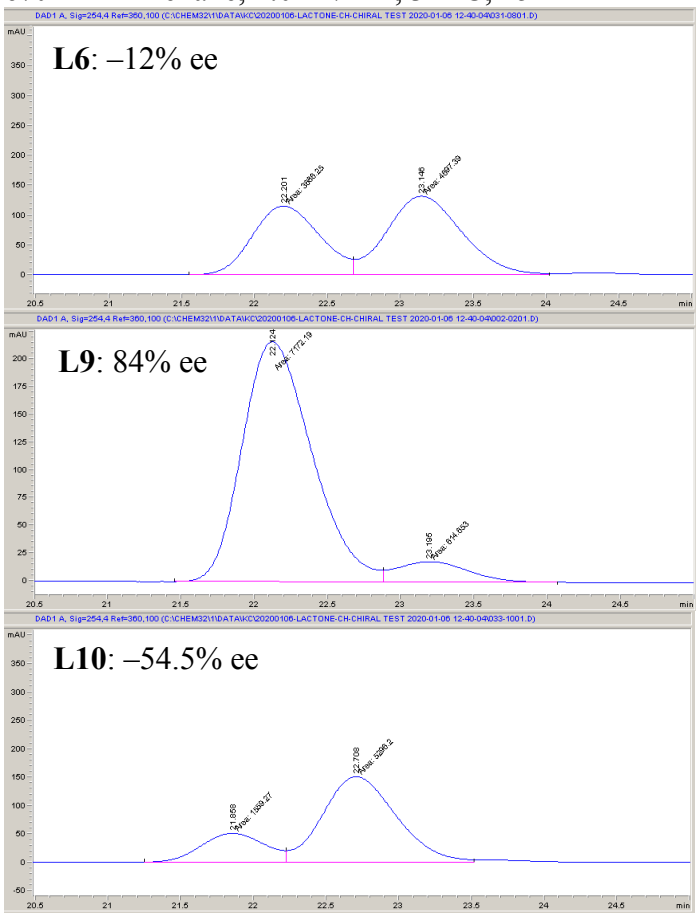
L6	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/µM	TTN	Avg.

OD_{600} = 60							TTN
2d-L6_a	2184.8	1198.0	1.8237	4.5611	3.57	1279	
2d-L6_b	2292.9	1197.8	1.9143	4.7876	3.57	1343	
2d-L6_c	2234.6	1204.8	1.8547	4.6387	3.57	1301	1308

L9 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2d-L9_a	3309.9	1166.7	2.8370	7.0953	5.18	1369	
2d-L9_b	3557.4	1191.0	2.9869	7.4702	5.18	1442	
2d-L9_c	3516.9	1195.6	2.9415	7.3568	5.18	1420	1410

L10 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2d-L10_a	975.1	1273.6	0.7656	1.9148	2.71	707	
2d-L10_b	992.8	1264.3	0.7853	1.9639	2.71	725	
2d-L10_c	979.1	1252.8	0.7815	1.9546	2.71	722	718

Chiral HPLC trace:
Chiralpak OD-H, 6% IPA in hexane, 1.0 ml/min, 32 °C, 254nm



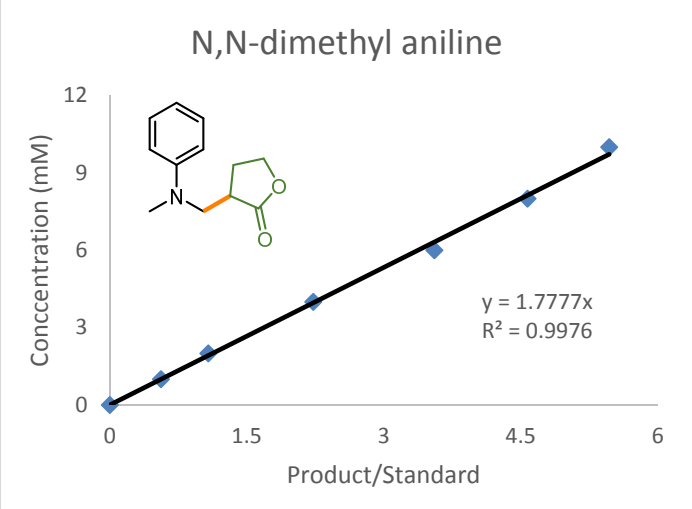
Area% report for enzymatically produced **2d**:

2d by variant **L6**			**2d** by variant **L9**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
22.20	3686.3	43.97	22.12	7172.2	92.11
23.15	4697.4	56.03	23.20	614.7	7.89
Total	8383.7	100.00	Total	7786.9	100.00

2d by variant **L10**		
Retention Time (min)	Area (mAU*s)	Area %
21.86	1559.3	22.75
22.71	5296.2	77.25
Total	6855.5	100.00

3-((Methyl(phenyl)amino)methyl)dihydrofuran-2(3*H*)-one (2e)

HPLC calibration curve (PhEA as internal standard):



Analysis Data:

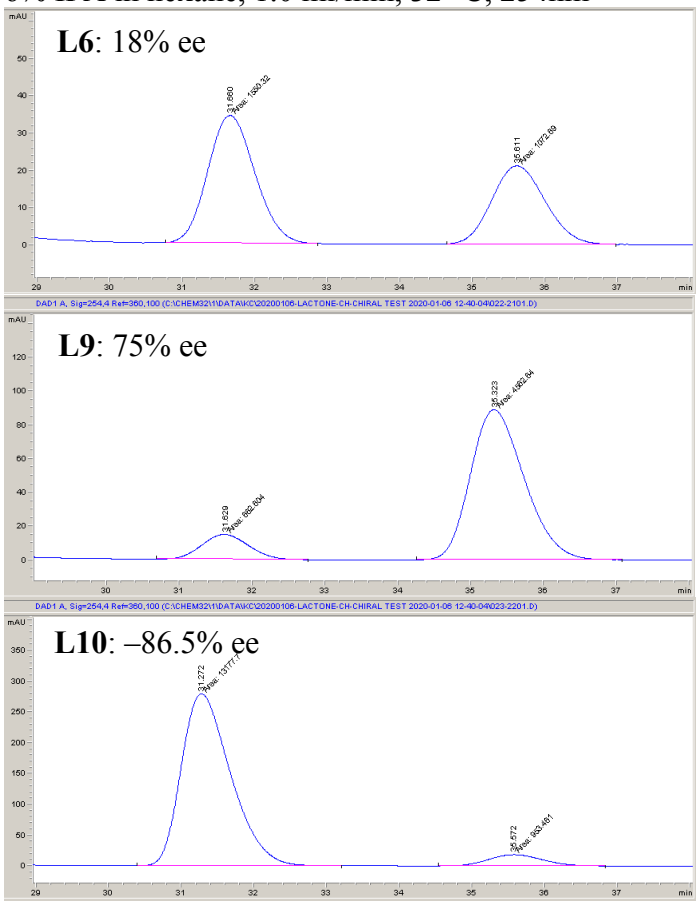
L6 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2e-L6_a	2614.2	2092.9	1.2491	2.2205	3.57	623	
2e-L6_b	2254.9	2094.7	1.0765	1.9137	3.57	537	
2e-L6_c	2440.0	2083.2	1.1713	2.0822	3.57	584	581

L9 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2e-L9_a	2284.6	2141.1	1.0670	1.8968	5.18	366	
2e-L9_b	2652.9	2066.7	1.2836	2.2819	5.18	440	
2e-L9_c	2555.8	2037.2	1.2546	2.2302	5.18	430	412

L10	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg.

OD_{600} = 60							TTN
2e-L10_a	5067.8	2157.2	2.3492	4.1763	2.71	1542	
2e-L10_b	5809.1	2190.8	2.6516	4.7137	2.71	1741	
2e-L10_c	5639.4	2190.4	2.5746	4.5769	2.71	1690	1658

Chiral HPLC trace:
Chiralpak OD-H, 6% IPA in hexane, 1.0 ml/min, 32 °C, 254nm



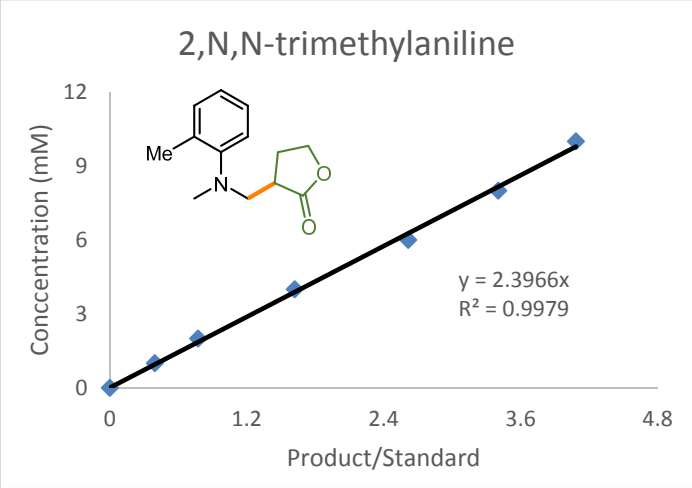
Area% report for enzymatically produced **2e**:

2e by variant **L6**			**2e** by variant **L9**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
31.66	1550.3	59.10	31.63	662.6	12.68
35.61	1072.7	40.90	35.32	4562.6	87.32
Total	2623.0	100.00	Total	5225.2	100.00

2e by variant **L10**		
Retention Time (min)	Area (mAU*s)	Area %
31.27	13177.7	93.25
35.57	953.5	6.75
Total	14131.2	100.00

3-((Methyl(*o*-tolyl)amino)methyl)dihydrofuran-2(3*H*)-one (2f)

HPLC calibration curve (*p*Me-anisole as internal standard):

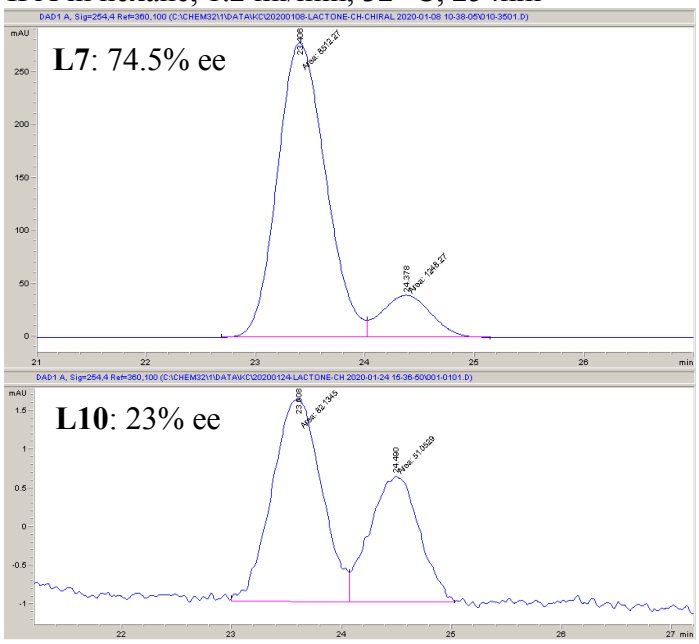


Analysis Data:

L9 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/µM	TTN	Avg. TTN
2f-L7_a	4037.9	1292.2	3.1248	7.4890	5.18	1445	
2f-L7_b	4090.4	1278.3	3.1999	7.6688	5.18	1480	
2f-L7_c	4210.4	1286.0	3.2740	7.8465	5.18	1514	1480

Chiral HPLC trace:

Chiralpak IC, 6% IPA in hexane, 1.2 ml/min, 32 °C, 254nm



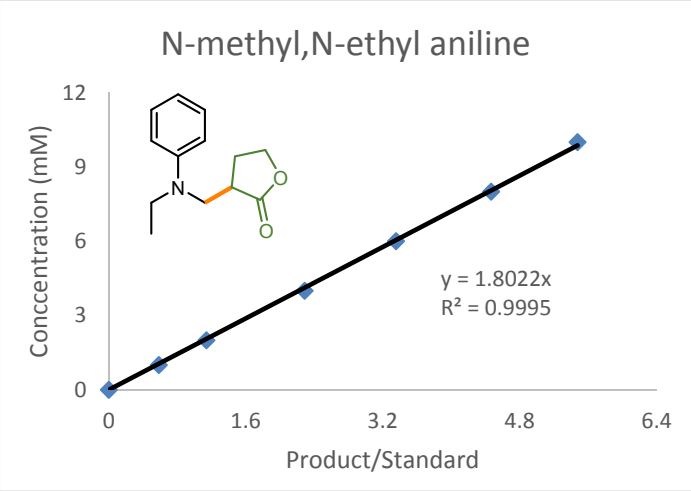
Area% report for enzymatically produced **2f**:

2f by variant **L9**	**2f** by variant **L10**

Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
23.41	8512.3	87.21	23.61	82.1	61.64
24.38	1248.3	12.79	24.49	51.1	38.36
Total	9760.6	100.00	Total	133.2	100.00

Note: Variant **L10** is not able to give good activity or reverse the enantioselectivity (compared to that with **L9**) when using substrates bearing an *ortho*-substituent on the phenyl ring (*e.g.*, substrates **1f** and **1k**).

3-((Ethyl(pheny)amino)methyl)dihydrofuran-2(3*H*)-one (2g)
3-(1-(Methyl(phenyl)amino)ethyl)dihydrofuran-2(3*H*)-one (2g')
HPLC calibration curve (*p*Me-anisole as internal standard):



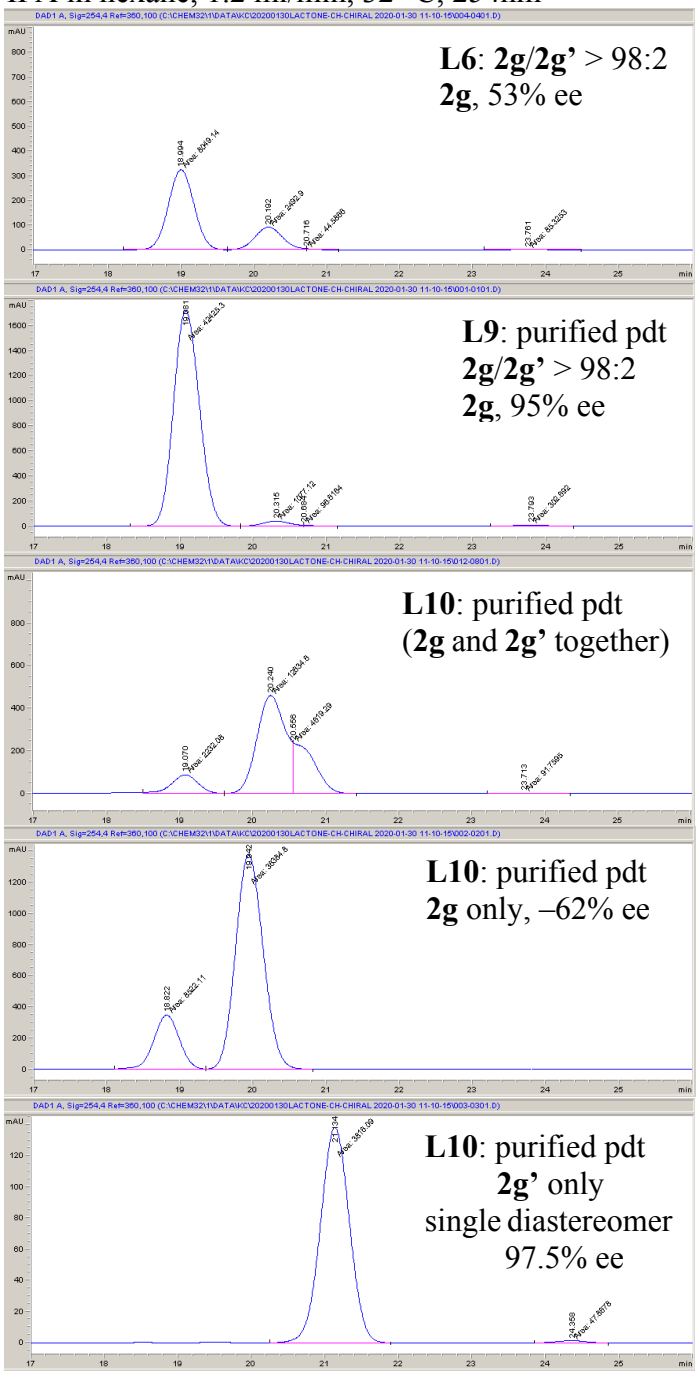
Analysis Data:

L6 OD_{600} = 60	Pdt-**2g**	Pdt-**2g'**	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2g/g'-L6_a	2871.8	77.7	1247.7	2.3639	4.2603	3.57	1195	
2g/g'-L6_b	2911.5	79.1	1241.2	2.4094	4.3423	3.57	1218	
2g/g'-L6_c	2889.4	80.9	1253.4	2.3698	4.2708	3.57	1198	1203

L9 OD_{600} = 60	Pdt-**2g**	Pdt-**2g'**	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2g/g'-L9_a	6070.1	84.9	1250.8	4.9209	8.8684	5.18	1711	
2g/g'-L9_b	6498.3	84.5	1292.6	5.0927	9.1780	5.18	1771	
2g/g'-L9_c	6185.2	85.3	1323.9	4.7364	8.5359	5.18	1647	1710

L10 OD_{600} = 60	Pdt-**2g**	Pdt-**2g'**	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2g/g'-L10_a	1606.2	672.3	1414.3	1.6110	2.9034	2.71	1072	
2g/g'-L10_b	1701.5	712.6	1404.6	1.7187	3.0975	2.71	1144	
2g/g'-L10_c	1698.3	712.0	1395.3	1.7274	3.1132	2.71	1150	1122

Chiral HPLC trace:
Chiralpak IC, 8% IPA in hexane, 1.2 ml/min, 32 °C, 254nm



Area% report for enzymatically produced **2g** and **2g'**:

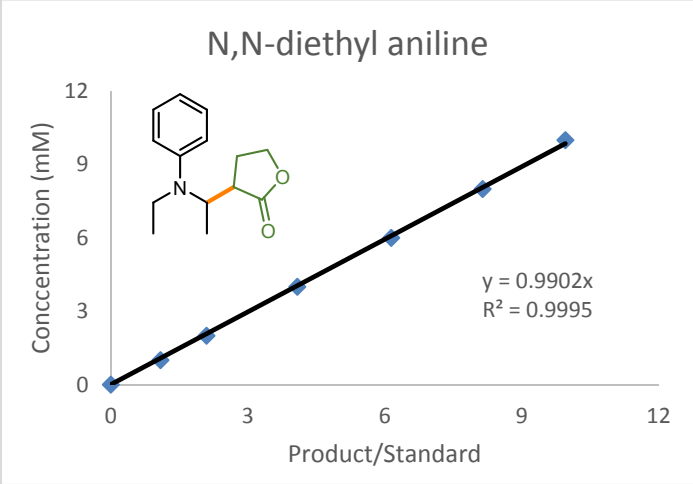
2g by variant **L6**			**2g** by variant **L9**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
18.99	8049.1	76.35	19.08	42425.3	97.52
20.19	2492.9	23.65	20.32	1077.1	2.48

Total	10542.0	100.00	Total	43502.4	100.00
2g by variant **L10**			**2g'** by variant **L10**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
18.82	8522.1	18.98	21.13	3816.1	98.76
19.94	36384.8	81.02	24.36	47.9	1.24
Total	44906.9	100.00	Total	3864	100.00

Note: The ee of **2g'** with variants **L6** and **L9** here cannot be accurately determined due to the peak overlap between one enantiomer of **2g** and one enantiomer of **2g'** (which is also very obvious in the situation of **L10**). Silica chromatography partially separated **2g** and **2g'**, which allowed for the ee determination of **2g** and **2g'** with **L10**. Additionally, the confirmed side product **2g''** had no effect on the ee determination of **2g** and **2g'** due to a very different retention time on chiral HPLC.

3-(1-(Ethyl(phenyl)amino)ethyl)dihydrofuran-2(3*H*)-one (2h)

HPLC calibration curve (PhEA as internal standard):



Analysis Data:

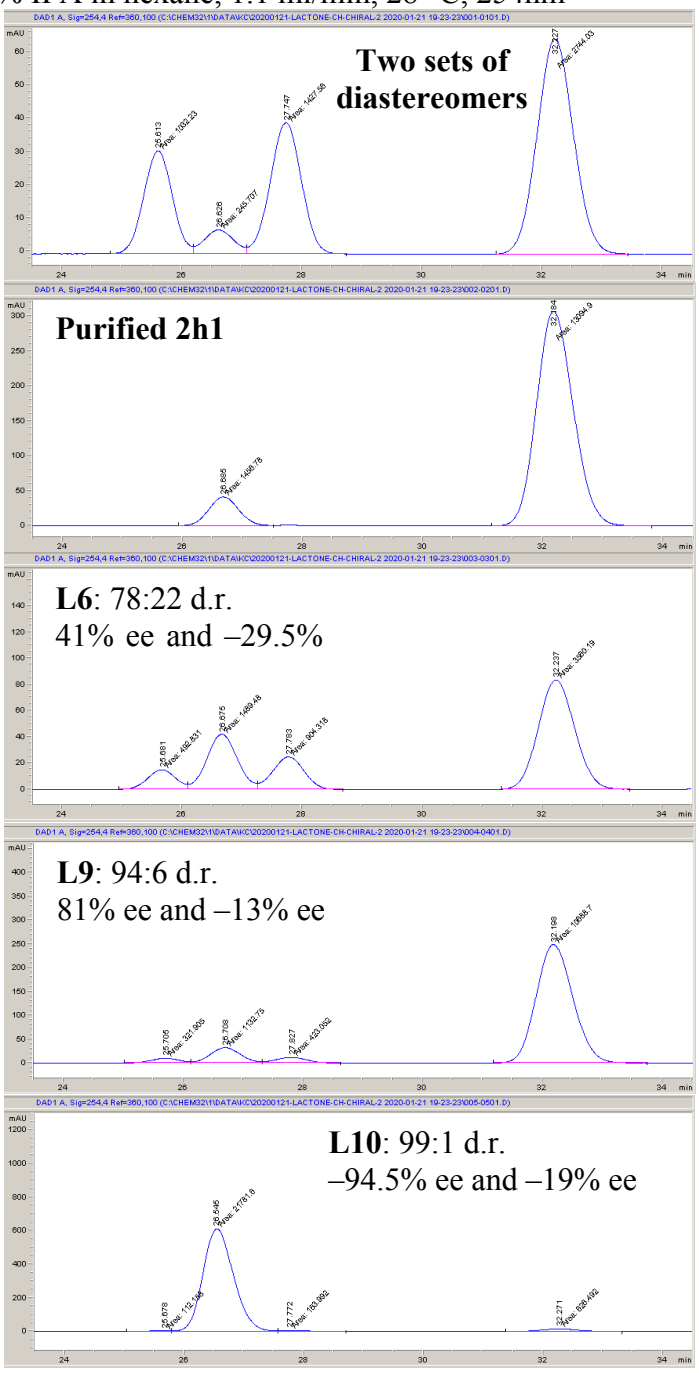
L6 OD_{600} = 60	Pdt-**2h1**	Pdt-**2h2**	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2h-L6_a	3483.2	984.7	2133.1	2.0946	2.0740	3.57	582	
2h-L6_b	3664.9	1031.0	2107.2	2.2285	2.2067	3.57	619	
2h-L6_c	3645.1	1021.7	2127.3	2.1938	2.1723	3.57	609	603

L9 OD_{600} = 60	Pdt-**2h1**	Pdt-**2h2**	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2h-L9_a	8696.2	583.0	2114.0	4.3894	4.3464	5.18	839	
2h-L9_b	8734.5	572.4	2112.3	4.4061	4.3629	5.18	842	
2h-L9_c	9017.8	591.4	2112.6	4.5485	4.5039	5.18	869	850

L10	Pdt-	Pdt-	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg.

OD_{600} = 60	**2h1**	**2h2**						TTN
2h-L10_a	4435.5	56.4	2218.1	2.0251	2.0053	2.71	740	
2h-L10_b	4492.9	59.2	2209.8	2.0600	2.0398	2.71	753	
2h-L10_c	4288.2	56.5	2211.3	1.9648	1.9455	2.71	718	737

Chiral HPLC trace:
Chiralpak IC, 4.5% IPA in hexane, 1.1 ml/min, 28 °C, 254nm

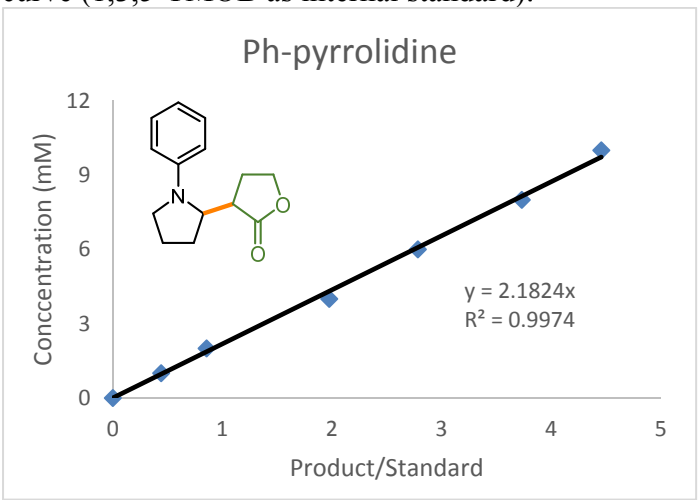


Area% report for enzymatically produced **2h1** and **2h2**:

2h1 by variant **L6**			**2h2** by variant **L6**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
26.68	1489.5	29.50	25.68	492.8	35.27
32.24	3560.2	70.50	27.78	904.3	64.73
Total	5049.7	100.00	Total	1397.1	100.00
2h1 by variant **L9**			**2h2** by variant **L9**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
26.71	1132.8	9.58	25.71	321.9	43.27
32.20	10688.7	90.42	27.83	423.1	56.73
Total	11821.5	100.00	Total	744.0	100.00
2h1 by variant **L10**			**2h2** by variant **L10**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
26.55	21781.6	97.20	25.68	112.2	40.62
32.27	626.5	2.80	27.77	164.0	59.38
Total	22408.1	100.00	Total	276.2	100.00

3-(1-Phenylpyrrolidin-2-yl)dihydrofuran-2(3*H*)-one (2i)

HPLC calibration curve (1,3,5-TMOB as internal standard):



Analysis Data:

L6 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2i-L6_a	5487.8	1314.6	4.1745	9.1104	3.57	2555	
2i-L6_b	5440.1	1301.8	4.1789	9.1200	3.57	2558	
2i-L6_c	5450.8	1308.0	4.1673	9.0947	3.57	2550	2554

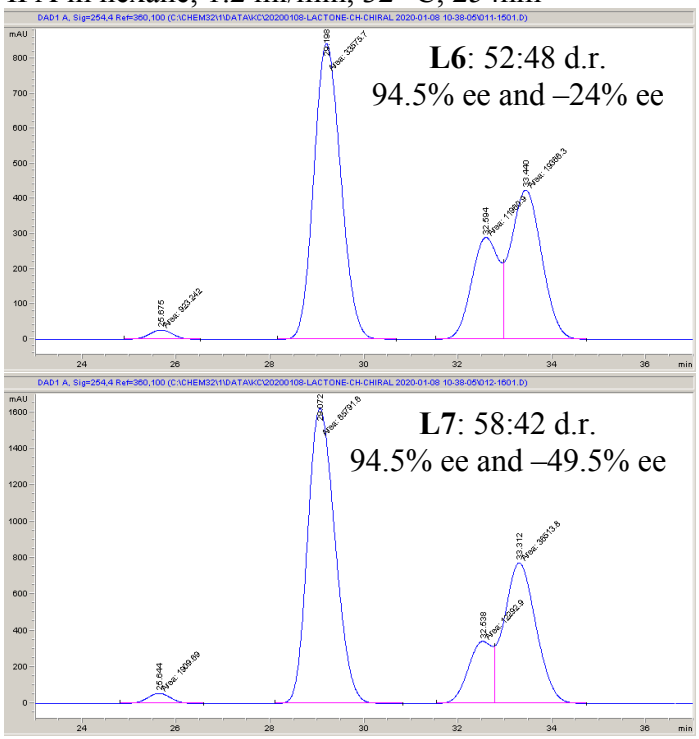
L7 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN

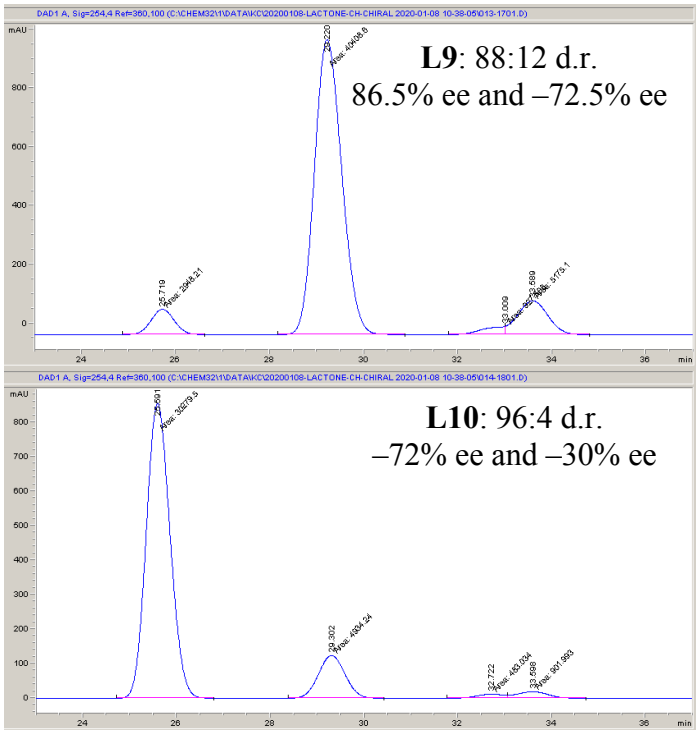
2i-L7_a	6040.7	1293.6	4.6697	10.1911	8.80	1157	
2i-L7_b	6051.7	1295.3	4.6720	10.1963	8.80	1158	
2i-L7_c	6017.4	1281.8	4.6945	10.2453	8.80	1164	1160

L9 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2i-L9_a	4648.3	1371.8	3.3885	7.3950	5.27	1404	
2i-L9_b	4805.3	1370.5	3.5062	7.6520	5.27	1453	
2i-L9_c	4844.6	1402.7	3.4538	7.5375	5.27	1431	1429

L10 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2i-L10_a	2388.6	1356.8	1.7605	3.8420	2.71	1419	
2i-L10_b	2507.7	1307.3	1.9182	4.1863	2.71	1546	
2i-L10_c	2576.4	1373.3	1.8761	4.0943	2.71	1512	1492

Chiral HPLC trace:
Chiralpak IC, 6% IPA in hexane, 1.2 ml/min, 32 °C, 254nm



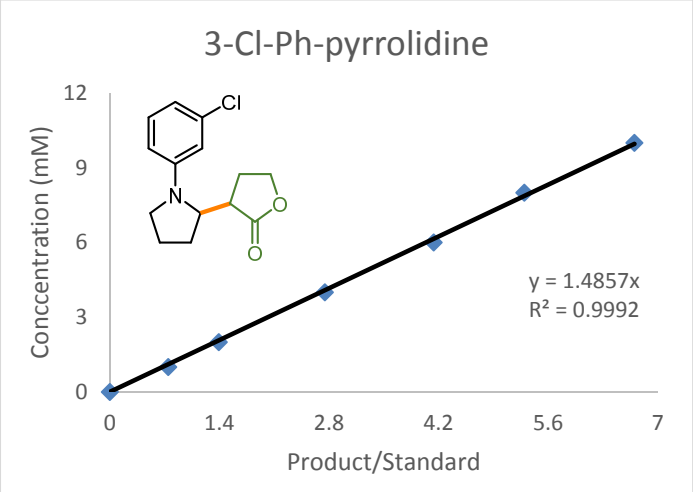


Area% report for enzymatically produced **2i1** and **2i2**:

2i1 by variant **L6**			**2i2** by variant **L6**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
25.68	923.2	2.68	32.59	11960.9	38.18
29.20	33575.7	97.32	33.44	19366.3	61.82
Total	34498.9	100.00	Total	31327.2	100.00
2i1 by variant **L7**			**2i2** by variant **L7**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
25.64	1909.7	2.82	32.54	12292.9	25.19
29.07	65791.6	97.18	33.31	36513.8	74.81
Total	67701.3	100.00	Total	48806.7	100.00
2i1 by variant **L9**			**2i2** by variant **L9**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
25.71	2948.2	6.80	33.01	827.6	13.79
29.22	40408.6	93.20	33.59	5175.1	86.21
Total	43356.8	100.00	Total	6002.7	100.00
2i1 by variant **L10**			**2i2** by variant **L10**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
25.59	30279.5	85.99	32.72	483.0	34.87
29.30	4934.2	14.01	33.60	902.0	65.13
Total	35213.7	100.00	Total	1385.0	100.00

3-(1-(3-Chlorophenyl)pyrrolidin-2-yl)dihydrofuran-2(3*H*)-one (2j)

HPLC calibration curve (*p*Me-anisole as internal standard):



Analysis Data:

L6 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2j-L6_a	3072.0	1337.8	2.2963	3.4116	3.45	990	
2j-L6_b	2853.6	1324.3	2.1548	3.2014	3.45	929	
2i-L6_c	2911.2	1312.4	2.2182	3.2956	3.45	957	959

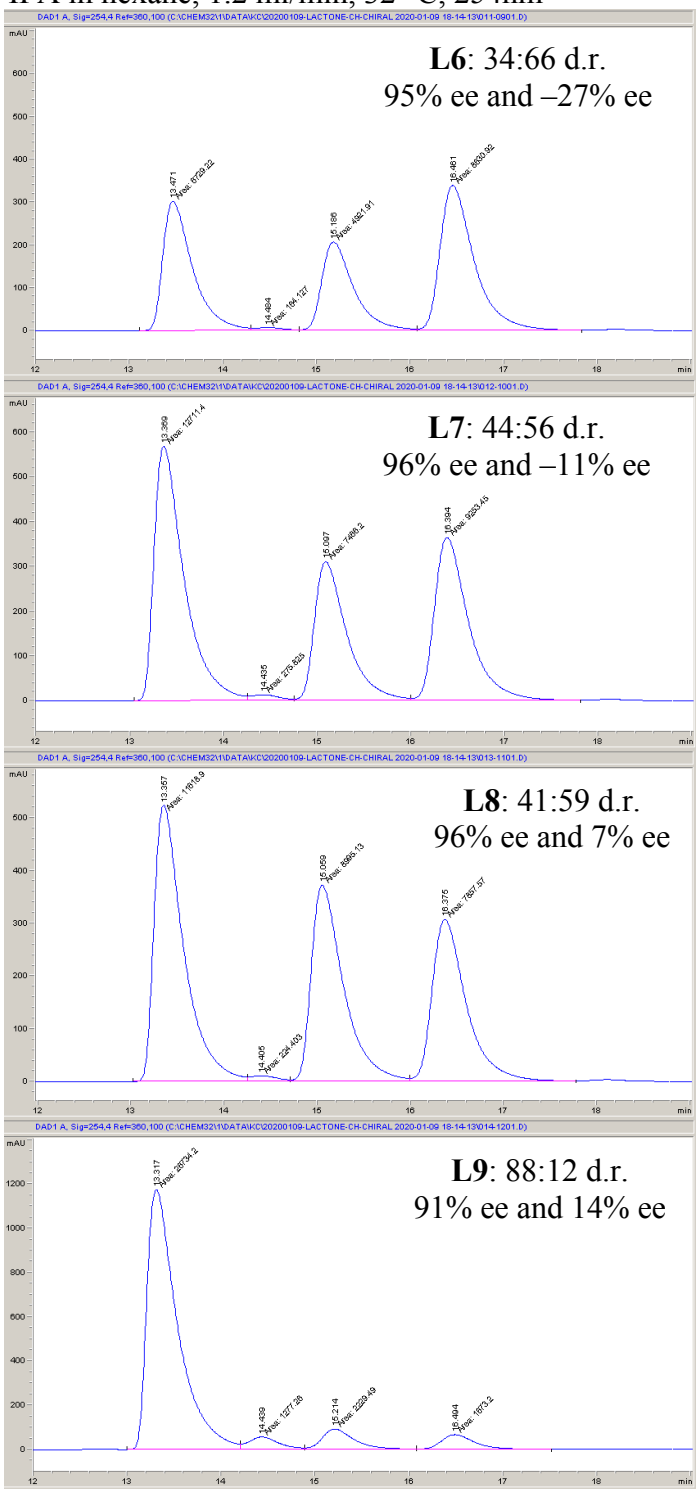
L7 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2j-L7_a	4395.6	1188.0	3.7000	5.4971	8.80	624	
2j-L7_b	3527.1	1067.0	3.3056	4.9112	8.80	558	
2j-L7_c	5181.6	1248.0	4.1519	6.1685	8.80	701	628

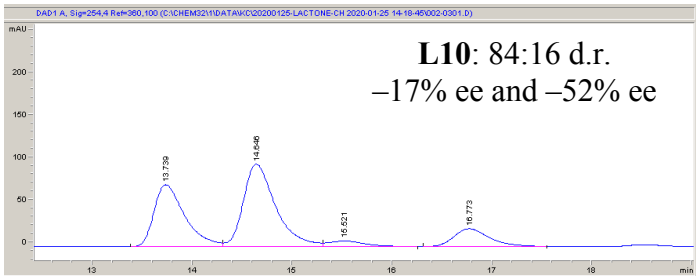
L8 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2j-L8_a	4296.0	1189.8	3.6107	5.3644	6.43	834	
2j-L8_b	4214.8	1189.6	3.5430	5.2639	6.43	819	
2j-L8_c	4289.5	1180.8	3.6327	5.3971	6.43	839	
2j-L8_d	4790.6	1193.6	4.0136	5.9630	6.43	927	855

L9 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2j-L9_a	5079.1	1171.5	4.3356	6.4413	5.19	1241	
2j-L9_b	6388.1	1177.2	5.4265	8.0622	5.19	1554	
2j-L9_c	5941.2	1160.0	5.1217	7.6093	5.19	1466	
2j-L9_d	6305.6	1154.1	5.4637	8.1173	5.19	1564	1456

Chiral HPLC trace:

Chiralpak IA, 3% IPA in hexane, 1.2 ml/min, 32 °C, 254nm

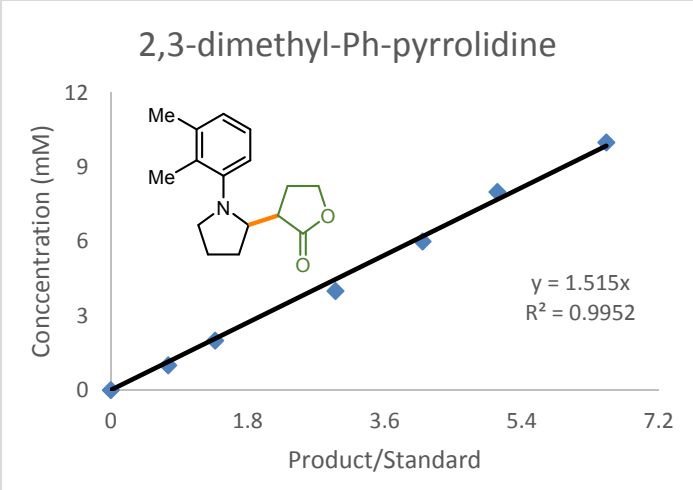




Area% report for enzymatically produced **2j1** and **2j2**:

2j1 by variant **L6**			**2j2** by variant **L6**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
13.47	6729.2	97.62	15.17	4921.9	36.32
14.48	164.1	2.38	16.46	8630.9	63.68
Total	6893.3	100.00	Total	13552.8	100.00
2j1 by variant **L7**			**2j2** by variant **L7**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
13.37	12711.4	97.88	15.10	7466.2	44.66
14.44	275.8	2.12	16.39	9253.5	55.34
Total	12987.2	100.00	Total	16719.7	100.00
2j1 by variant **L8**			**2j2** by variant **L8**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
13.36	11618.9	98.11	15.06	8995.1	53.37
14.41	224.4	1.89	16.38	7857.6	46.63
Total	11843.3	100.00	Total	16852.7	100.00
2j1 by variant **L9**			**2j2** by variant **L9**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
13.32	26734.2	95.44	15.21	2229.5	57.13
14.44	1277.3	4.56	16.49	1673.2	42.87
Total	28011.5	100.00	Total	3902.7	100.00
2j1 by variant **L10**			**2j2** by variant **L10**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
13.74	1563.6	41.66	15.52	168.3	23.88
14.65	2189.9	58.34	16.77	536.6	76.12
Total	3753.5	100.00	Total	704.9	100.00

3-(1-(2,3-Dimethylphenyl)pyrrolidin-2-yl)dihydrofuran-2(3*H*)-one (2k)
HPLC calibration curve (*p*Me-anisole as internal standard):



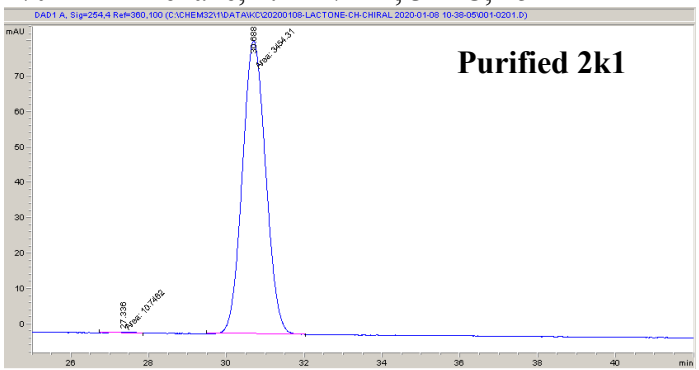
Analysis Data:

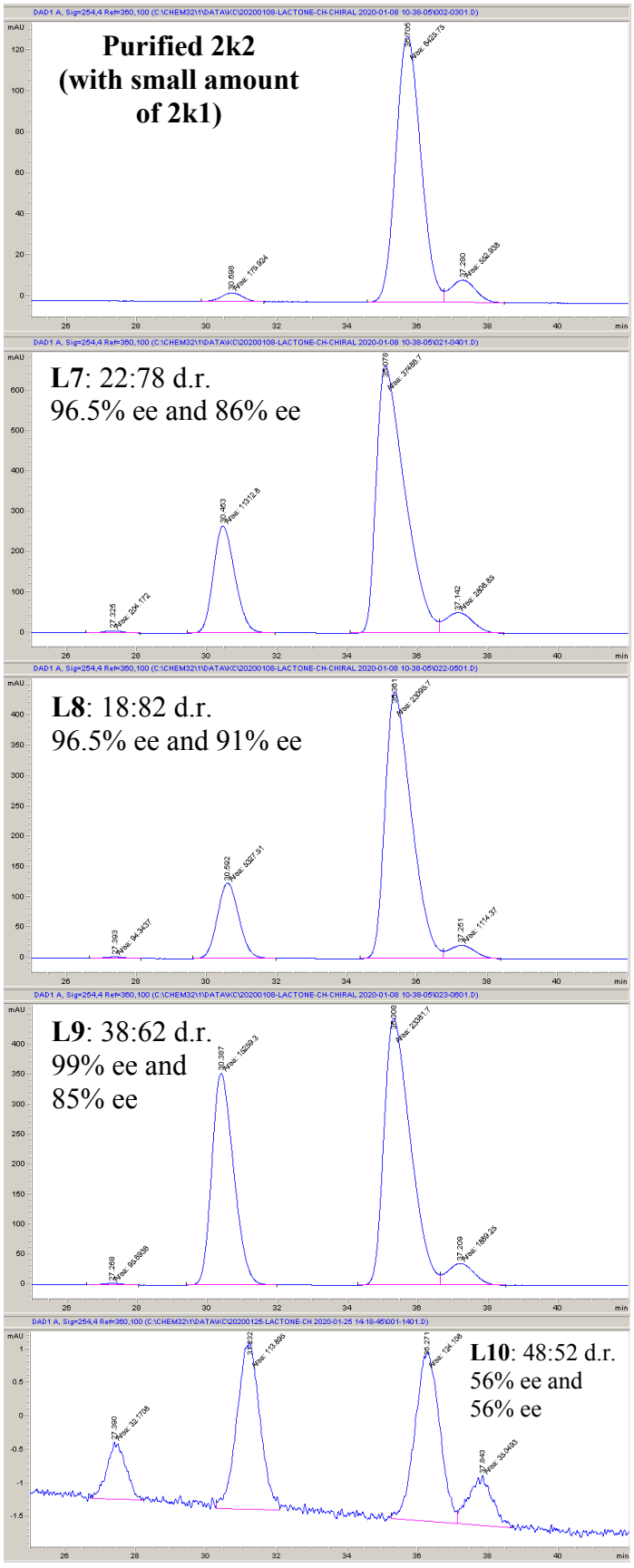
L7 OD_{600} = 30	Pdt-**2k1**	Pdt-**2k2**	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2k-L7_a	6348.1	1742.6	1185.9	6.8224	10.3360	4.40	2348	
2k-L7_b	6633.2	1820.7	1210.0	6.9867	10.5848	4.40	2404	
2k-L7_c	6673.6	1839.2	1197.0	7.1118	10.7743	4.40	2447	2400

L8 OD_{600} = 30	Pdt-**2k1**	Pdt-**2k2**	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2k-L8_a	5871.2	1294.8	1138.0	6.2970	9.5400	3.51	2716	
2k-L8_b	5708.7	1247.3	1152.8	6.0340	9.1415	3.51	2602	
2k-L8_c	5912.4	1280.9	1132.5	6.3517	9.6228	3.51	2739	
2k-L8_d	6284.3	1359.8	1149.4	6.6505	10.0755	3.51	2868	2731

L9 OD_{600} = 30	Pdt-**2k1**	Pdt-**2k2**	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2k-L9_a	5705.5	3229.5	1257.1	7.1076	10.7681	2.63	4089	
2k-L9_b	5650.4	3192.2	1286.2	6.8750	10.4156	2.63	3955	
2k-L9_c	5418.3	3073.9	1233.8	6.8830	10.4277	2.63	3959	4001

Chiral HPLC trace:
Chiralpak OD-H, 4% IPA in hexane, 1.2 ml/min, 32 °C, 254nm



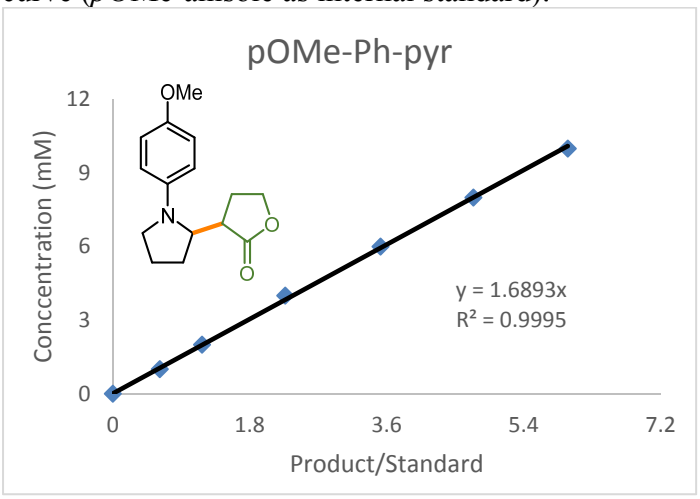

Purified 2k2
(with small amount
of 2k1)
L7: 22:78 d.r.
96.5% ee and 86% ee
L8: 18:82 d.r.
96.5% ee and 91% ee
L9: 38:62 d.r.
99% ee and
85% ee
L10: 48:52 d.r.
56% ee and
56% ee

Area% report for enzymatically produced **2k1** and **2k2**:

2k1 by variant **L7**			**2k2** by variant **L7**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
27.33	204.2	1.77	35.08	37486.7	93.03
30.45	11312.8	98.23	37.14	2808.9	6.97
Total	11517.0	100.00	Total	40295.6	100.00
2k1 by variant **L8**			**2k2** by variant **L8**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
27.39	94.3	1.74	35.36	23095.7	95.40
30.59	5327.5	98.26	37.25	1114.4	4.60
Total	5421.8	100.00	Total	24210.1	100.00
2k1 by variant **L9**			**2k2** by variant **L9**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
27.27	95.7	0.62	35.31	23381.7	92.52
30.39	15259.3	99.38	37.21	1889.3	7.48
Total	15355.0	100.00	Total	25271.0	100.00
2k1 by variant **L10**			**2k2** by variant **L10**		
Retention Time (min)	Area (mAU*s)	Area %	Retention Time (min)	Area (mAU*s)	Area %
27.39	32.2	22.04	36.27	124.1	78.00
31.23	113.9	77.96	37.84	35.0	22.00
Total	146.1	100.00	Total	159.1	100.00

3-(1-(4-Methoxyphenyl)pyrrolidin-2-yl)dihydrofuran-2(3*H*)-one (2l)

HPLC calibration curve (*p*OMe-anisole as internal standard):



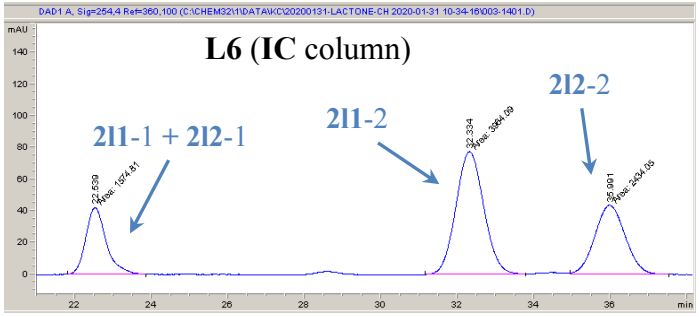
Analysis Data:

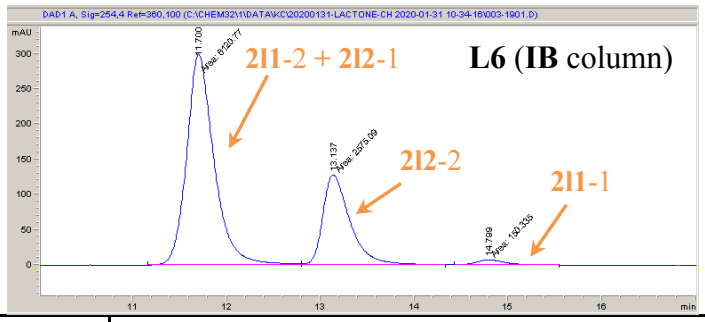
L6 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2l-L6_a	2160.9	1152.1	1.8756	3.1685	3.35	947	
2l-L6_b	2200.0	1141.9	1.9266	3.2546	3.35	973	
2l-L6_c	2174.0	1179.3	1.8435	3.1142	3.35	931	
2l-L6_d	2197.9	1142.6	1.9236	3.2495	3.35	971	955

L7 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2l-L7_a	6904.9	1125.9	6.1328	10.3601	8.20	1263	
2l-L7_b	7614.6	1117.4	6.8146	11.5119	8.20	1403	
2l-L7_c	6405.5	1118.7	5.7258	9.6727	8.20	1179	
2l-L7_d	6480.6	1116.2	5.8059	9.8080	8.20	1196	1260

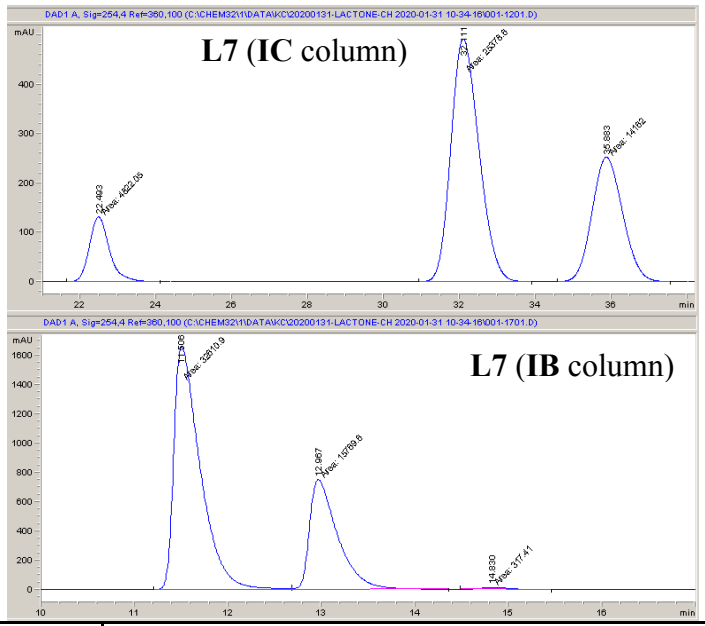
L9 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2l-L9_a	657.9	1213.1	0.5423	0.9162	4.55	201	
2l-L9_b	658	1179.6	0.5578	0.9423	4.55	207	
2l-L9_c	667.2	1189.6	0.5609	0.9475	4.55	208	
2l-L9_d	679.7	1170.3	0.5808	0.9811	4.55	216	208

Chiral HPLC trace:
Chiralpak IC, 25% IPA in hexane, 1.2 ml/min, 28 °C, 254nm
Chiralpak IB, 12% IPA in hexane, 1.2 ml/min, 28 °C, 254nm
(Note: The four stereo-isomers of product **2l** could not be fully separated by chiral HPLC columns. We thus used two different columns to have three sets of peaks each, as labelled in the HPLC traces, and then calculated the enantiomeric excess according to the peak ratios).

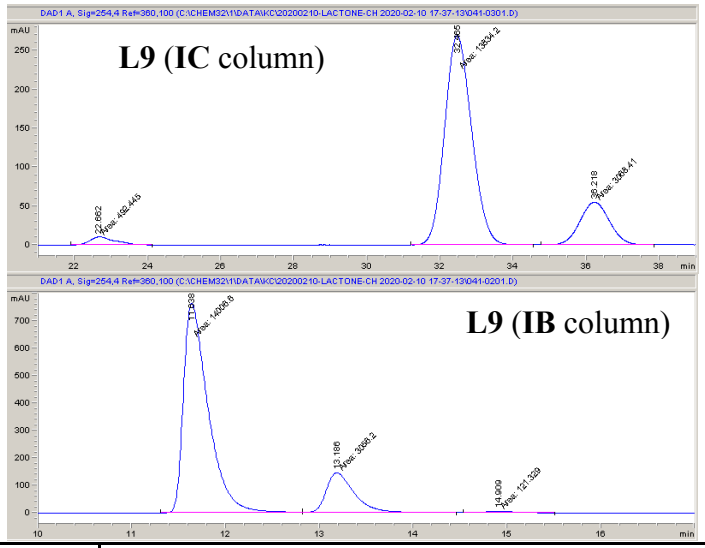




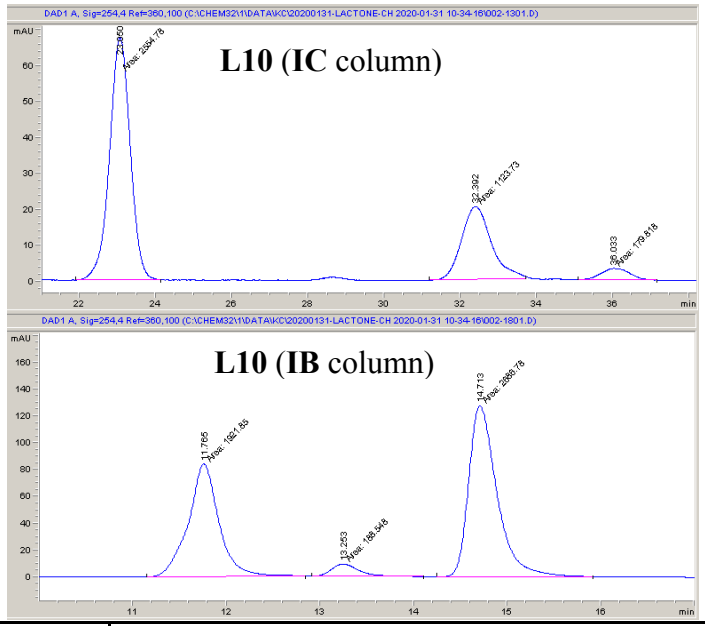
2l	peak-1	peak-2	peak-3	sum
L6-IC	1574.8	3964.1	2434.1	7973
	19.75%	49.72%	30.53%	100.00%
L6-IB	6120.8	2575.1	150.3	8846.2
	69.19%	29.11%	1.70%	100.00%
	2l1-1	**2l1**-2	**2l2**-1	**2l2**-2
	1.70%	50.43%	18.05%	29.82%
52:48 d.r.	**2l1** 93% ee		**2l2** –26% ee	



2l	peak-1	peak-2	peak-3	sum
L7-IC	4822.1	25378.6	14162	44362.7
	10.87%	57.21%	31.92%	100.00%
L7-IB	32610.9	15769.6	317.4	48697.9
	66.97%	32.38%	0.65%	100.00%
	2l1-1	**2l1**-2	**2l2**-1	**2l2**-2
	0.65%	56.98%	10.22%	32.15%
58:42 d.r.	**2l1** 98% ee		**2l2** –51.5% ee	



2l	peak-1	peak-2	peak-3	sum
L9-IC	492.4	13834.2	3068.4	17395
	2.83%	79.53%	17.64%	100.00%
L9-IB	14006.6	3056.2	121.3	17184.1
	81.51%	17.79%	0.71%	100.00%
	2l1-1	**2l1**-2	**2l2**-1	**2l2**-2
	0.71%	79.46%	2.12%	17.71%
80:20 d.r.	**2l1** 98% ee		**2l2** –78.5% ee	

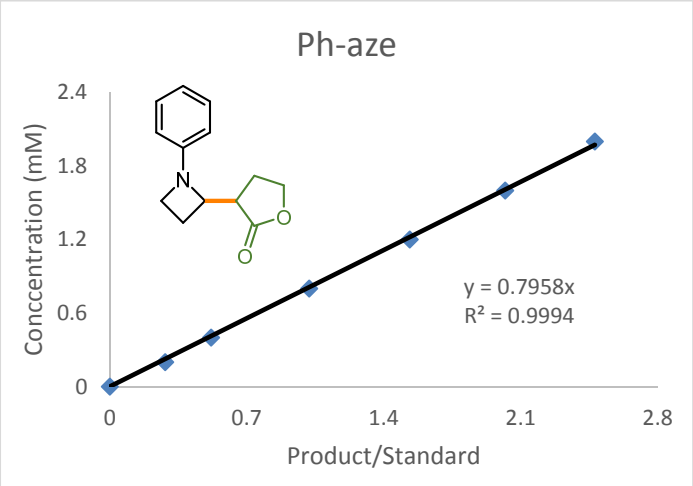


2l	peak-1	peak-2	peak-3	sum
L10-IC	2554.8	1123.7	179.8	3858.3
	66.22%	29.12%	4.66%	100.00%
L10-IB	1921.9	188.5	2686.8	4797.2

	40.06%	3.93%	56.01%	100.00%
	2l1-1	**2l1**-2	**2l2**-1	**2l2**-2
	56.01%	29.49%	10.21%	4.29%
80:20 d.r.	**2l1** –31% ee		**2l2** 37% ee	

3-(1-Phenylazetidin-2-yl)dihydrofuran-2(3*H*)-one (2m)

HPLC calibration curve (AllylOPh as internal standard):

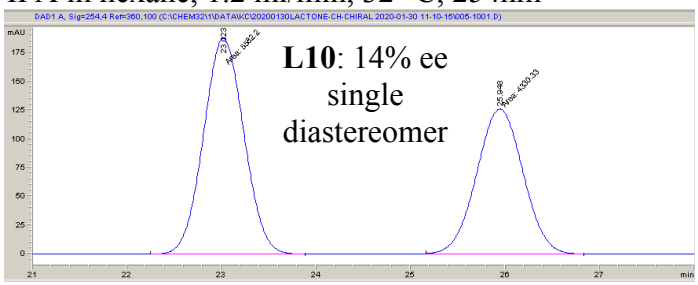


Analysis Data:

L10 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2m-L10_a	374.2	760.4	0.4921	0.3916	2.71	145	
2m-L10_b	374.3	755.3	0.4956	0.3944	2.71	146	
2m-L10_c	360.4	760.7	0.4738	0.3770	2.71	139	143

Chiral HPLC trace:

Chiralpak IC, 8% IPA in hexane, 1.2 ml/min, 32 °C, 254nm



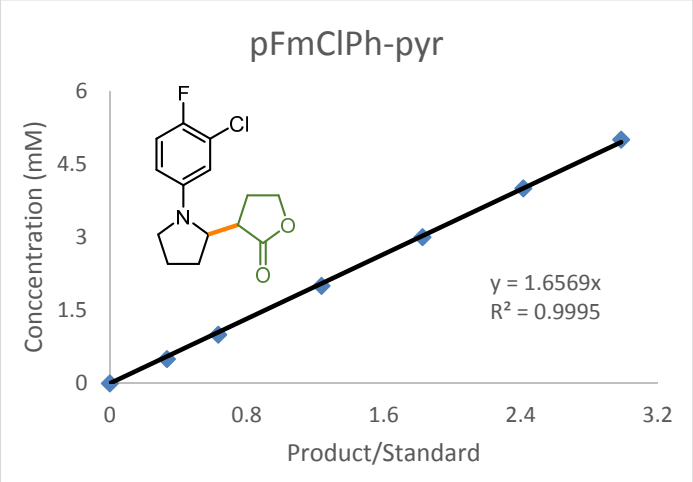
Area% report for enzymatically produced **2m**:

2m by variant **L10**		
Retention Time (min)	Area (mAU*s)	Area %
23.02	5582.2	56.31

25.95	4330.3	43.69
Total	9912.5	100.00

3-(1-(3-Chloro-4-fluorophenyl)pyrrolidin-2-yl)dihydrofuran-2(3*H*)-one (2n)

HPLC calibration curve (1,3,5-TMOB as internal standard):

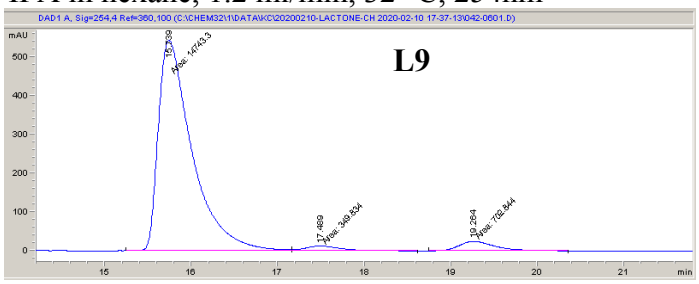


Analysis Data:

L9 OD_{600} = 60	Pdt	Std	Pdt/Std	[Pdt]/mM	[PC]/μM	TTN	Avg. TTN
2n-L9_a	2025.3	1375.6	1.4723	2.4395	5.27	463	
2n-L9_b	1834.4	1379.1	1.3301	2.2039	5.27	418	
2n-L9_c	1841.7	1367.4	1.3469	2.2316	5.27	424	435

Chiral HPLC trace:

Chiralpak IA, 3% IPA in hexane, 1.2 ml/min, 32 °C, 254nm



Note: Only three peaks were observed and confirmed to belong to the stereo-isomers of product **2n**. No conclusion on ee should be made unless further analysis is carried out.

VI. Sequence Information

Mutations present in P411 variants involved in this study:

P411 variant	Mutations relative to wild-type P450$_{BM3}$
P411-**C10** (**L1**)	N70E A74G V78L A82L F87A M118S P142S F162L T175I M177L A184V S226R H236Q E252G I263Y H266V T268G A290V A328V A330Y L353V I366V C400S I401L T436L L437Q E442K ΔFAD*
L2	N70E A74G V78L A82L F87A M118S P142S F162L T175I M177L A184V S226R H236Q E252G I263Y H266V T268G A290V **T327V** A328V A330Y L353V I366V C400S I401L T436L L437Q E442K ΔFAD*
L3	N70E A74G V78L A82L F87A M118S P142S F162L T175I M177L A184V S226R H236Q E252G I263Y H266V T268G A290V **T327V** A328V A330Y L353V I366V C400S I401L T436L E442K ΔFAD*
L4	N70E A74G V78L A82L F87A M118S P142S F162L T175I M177L A184V S226R H236Q E252G I263Y H266V T268G A290V **T327V** A328V A330Y **S332A** L353V I366V C400S I401L T436L E442K ΔFAD*
L5	N70E A74G V78L A82L **F87P** M118S P142S F162L T175I M177L A184V S226R H236Q E252G I263Y H266V T268G A290V **T327V** A328V A330Y **S332A** L353V I366V C400S I401L T436L E442K ΔFAD*
L6	N70E A74G V78L A82L **F87P** M118S P142S F162L T175I M177L A184V S226R H236Q E252G I263Y **A264S** H266V T268G A290V **T327V** A328V A330Y **S332A** L353V I366V C400S I401L T436L E442K ΔFAD*
L7	N70E A74G V78L A82L **F87P** M118S P142S F162L T175I M177L A184V S226R H236Q E252G I263Y **A264S** H266V T268G A290V **T327P** A328V A330Y **S332A** L353V I366V C400S I401L T436L E442K ΔFAD*
L8	N70E A74G V78L A82L **F87P** M118S P142S F162L T175I M177L A184V S226R H236Q E252G I263Y **A264S** H266V **E267D** T268G A290V **T327P** A328V A330Y **S332A** L353V I366V C400S I401L T436L E442K ΔFAD*
L9	N70E A74G V78L A82L **F87P** M118S P142S F162L T175I M177L A184V S226R H236Q E252G I263Y **A264S** H266V **E267D** T268G A290V **T327P A328L** A330Y **S332A** L353V I366V C400S I401L T436L E442K ΔFAD*
L10	N70E A74G V78L A82L **F87P** M118S P142S F162L T175I M177L A184V S226R H236Q E252G I263Y **A264S** H266V **E267D** T268G A290V **T327P A328R** A330Y **S332A** L353V I366V C400S I401L T436L E442K ΔFAD*

*ΔFAD: FAD domain truncation.

Nucleotide and amino acid sequences of P411-C10:

ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCGTTATTAAA
CACAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGAGAAATCTTTAAATTCG
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Nucleotide and amino acid sequences of P411-L2:

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Nucleotide and amino acid sequences of P411-L3:

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Nucleotide and amino acid sequences of P411-L4:

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Nucleotide and amino acid sequences of P411-L5:

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GCTACTCCGTATTTGGATGCGGCGATAAAAACTGGGCTACTACGTATCAAAAAGTGCCTGCTTTT
ATCGATGAAACGCTTGCCGCTAAAGGGGCAGAAAACATCGCTGACCGCGGTGAAGCAGATGCAAG
CGACGACTTTGAAGGCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAGCAGCCTACT
TTAACCTCGACATTGAAAACAGTGAAGATAATAAATCTACTCTTTCACTTCAATTTGTCGACAGC
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SRFDKELSQGLKFLRDFLGDGL**P**TSWTHEKNWKKAHNILLPSFSQQAMKGYHASMVDIAVQLVQK
WERLNADEHIEVSEDMTRLTLDTIGLCGFNYRLNSFYRDQPHPFIISLVRALDEVMNKLQRANPD
DPAYDENKRQFQEDIKVMNDLVDKIIADRKARGEQSDDLLTQMLNGKDPETGEPLDDGNIRYQII
TFLYAGVEGTSGLLSFALYFLVKNPHVLQKVAEEAARVLVDPVPSYKQVKQLKYVGMVLNEALRL
WP**V**VPYF**A**LYAKEDTVLGGEYPLEKGDEVMVLIPQLHRDKTVWGDDVEEFRPERFENPSAIPQHA
FKPFGNGQRASLGQQFALHEATLVLGMMLKHFDFEDHTNYELDIKEL**L**TLKPKGFVVKAKSKKIP
LGGIPSPSTEQSAKKVRKKAENAHNTPLLVLYGSNMGTAEGTARDLADIAMSKGFAPQVATLDSH
AGNLPREGAVLIVTASYNGHPPDNAKQFVDWLDQASADEVKGVRYSVFGCGDKNWATTYQKVPAF
IDETLAAKGAENIADRGEADASDDFEGTYEEWREHMWSDVAAYFNLDIENSEDNKSTLSLQFVDS
AADMPLAKMHGAFSTLEHHHHHH

Nucleotide and amino acid sequences of P411-L6:

ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCGTTATTAAA
CACAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGAGAAATCTTTAAATTCG
AGGCGCCTGGTCGTGTAACGCGCTACTTATCAAGTCAGCGTCTAATTAAAGAAGCATGCGATGAA
TCACGCTTTGATAAAGAGTTAAGTCAAGGTCTGAAATTTCTGCGTGATTTTCTTGGAGACGGGTT
A**CCG**ACAAGCTGGACGCATGAAAAAAATTGGAAAAAAGCGCATAATATCTTACTTCCAAGCTTTA
GTCAGCAGGCAATGAAAGGCTATCATGCGAGTATGGTCGATATCGCCGTGCAGCTTGTTCAAAAG
TGGGAGCGTCTAAATGCAGATGAGCATATTGAAGTATCGGAAGACATGACACGTTTAACGCTTGA
TACAATTGGTCTTTGCGGCTTTAACTATCGCCTTAACAGCTTTTACCGAGATCAGCCTCATCCAT
TTATTATAAGTCTGGTCCGTGCACTGGATGAAGTAATGAACAAGCTGCAGCGAGCAAATCCAGAC
GACCCAGCTTATGATGAAAACAAGCGCCAGTTTCAAGAAGATATCAAGGTGATGAACGACCTAGT
AGATAAAATTATTGCAGATCGCAAAGCAAGGGGTGAACAAAGCGATGATTTATTAACGCAGATGC
TAAACGGAAAAGATCCAGAAACGGGTGAGCCGCTTGATGACGGGAACATTCGCTATCAAATTATT
ACATTCTTATAT**AGT**GGAGTTGAAGGTACAAGTGGTCTTTTATCATTTGCGCTGTATTTCTTAGT
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CAAGCTACAAACAAGTCAAACAGCTTAAATATGTCGGCATGGTCTTAAACGAAGCGCTGCGCTTA
TGGCCA**GTG**GTTCCTTATTTT**GCG**CTATATGCAAAAGAAGATACGGTGCTTGGAGGAGAATATCC
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GAGACGATGTGGAGGAGTTCCGTCCAGAGCGTTTTGAAAATCCAAGTGCGATTCCGCAGCATGCG
TTTAAACCGTTTGGAAACGGTCAGCGTGCGTCTCTGGGTCAGCAGTTCGCTCTTCATGAAGCAAC
GCTGGTACTTGGTATGATGCTAAAACACTTTGACTTTGAAGATCATACAAACTACGAGCTCGATA
TTAAAGAACTG**CTT**ACGTTAAAACCTAAAGGCTTTGTGGTAAAAGCAAAATCGAAAAAAATTCCG
CTTGGCGGTATTCCTTCACCTAGCACTGAACAGTCTGCTAAAAAAGTACGCAAAAAGGCAGAAAA
CGCTCATAATACGCCGCTGCTTGTGCTATACGGTTCAAATATGGGTACCGCTGAAGGAACGGCGC
GTGATTTAGCAGATATTGCAATGAGCAAAGGATTTGCACCGCAGGTCGCAACGCTTGATTCACAC
GCCGGAAATCTTCCGCGCGAAGGAGCTGTATTAATTGTAACGGCGTCTTATAACGGTCATCCGCC

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GCCGCGGATATGCCGCTTGCGAAAATGCACGGTGCGTTTTCAACGCTCGAGCACCACCACCACCA
CCACTGA

MTIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKEACDE
SRFDKELSQGLKFLRDFLGDGL**P**TSWTHEKNWKKAHNILLPSFSQQAMKGYHASMVDIAVQLVQK
WERLNADEHIEVSEDMTRLTLDTIGLCGFNYRLNSFYRDQPHPFIISLVRALDEVMNKLQRANPD
DPAYDENKRQFQEDIKVMNDLVDKIIADRKARGEQSDDLLTQMLNGKDPETGEPLDDGNIRYQII
TFLY**S**GVEGTSGLLSFALYFLVKNPHVLQKVAEEAARVLVDPVPSYKQVKQLKYVGMVLNEALRL
WP**V**VPYF**A**LYAKEDTVLGGEYPLEKGDEVMVLIPQLHRDKTVWGDDVEEFRPERFENPSAIPQHA
FKPFGNGQRASLGQQFALHEATLVLGMMLKHFDFEDHTNYELDIKEL**L**TLKPKGFVVKAKSKKIP
LGGIPSPSTEQSAKKVRKKAENAHNTPLLVLYGSNMGTAEGTARDLADIAMSKGFAPQVATLDSH
AGNLPREGAVLIVTASYNGHPPDNAKQFVDWLDQASADEVKGVRYSVFGCGDKNWATTYQKVPAF
IDETLAAKGAENIADRGEADASDDFEGTYEEWREHMWSDVAAYFNLDIENSEDNKSTLSLQFVDS
AADMPLAKMHGAFSTLEHHHHHH

Nucleotide and amino acid sequences of P411-L7:

ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCGTTATTAAA
CACAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGAGAAATCTTTAAATTCG
AGGCGCCTGGTCGTGTAACGCGCTACTTATCAAGTCAGCGTCTAATTAAAGAAGCATGCGATGAA
TCACGCTTTGATAAAGAGTTAAGTCAAGGTCTGAAATTTCTGCGTGATTTTCTTGGAGACGGGTT
A**CCG**ACAAGCTGGACGCATGAAAAAAATTGGAAAAAAGCGCATAATATCTTACTTCCAAGCTTTA
GTCAGCAGGCAATGAAAGGCTATCATGCGAGTATGGTCGATATCGCCGTGCAGCTTGTTCAAAAG
TGGGAGCGTCTAAATGCAGATGAGCATATTGAAGTATCGGAAGACATGACACGTTTAACGCTTGA
TACAATTGGTCTTTGCGGCTTTAACTATCGCCTTAACAGCTTTTACCGAGATCAGCCTCATCCAT
TTATTATAAGTCTGGTCCGTGCACTGGATGAAGTAATGAACAAGCTGCAGCGAGCAAATCCAGAC
GACCCAGCTTATGATGAAAACAAGCGCCAGTTTCAAGAAGATATCAAGGTGATGAACGACCTAGT
AGATAAAATTATTGCAGATCGCAAAGCAAGGGGTGAACAAAGCGATGATTTATTAACGCAGATGC
TAAACGGAAAAGATCCAGAAACGGGTGAGCCGCTTGATGACGGGAACATTCGCTATCAAATTATT
ACATTCTTATAT**AGT**GGAGTTGAAGGTACAAGTGGTCTTTTATCATTTGCGCTGTATTTCTTAGT
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CAAGCTACAAACAAGTCAAACAGCTTAAATATGTCGGCATGGTCTTAAACGAAGCGCTGCGCTTA
TGGCCA**CCG**GTTCCTTATTTT**GCG**CTATATGCAAAAGAAGATACGGTGCTTGGAGGAGAATATCC
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TTTAAACCGTTTGGAAACGGTCAGCGTGCGTCTCTGGGTCAGCAGTTCGCTCTTCATGAAGCAAC
GCTGGTACTTGGTATGATGCTAAAACACTTTGACTTTGAAGATCATACAAACTACGAGCTCGATA
TTAAAGAACTG**CTT**ACGTTAAAACCTAAAGGCTTTGTGGTAAAAGCAAAATCGAAAAAAATTCCG
CTTGGCGGTATTCCTTCACCTAGCACTGAACAGTCTGCTAAAAAAGTACGCAAAAAGGCAGAAAA
CGCTCATAATACGCCGCTGCTTGTGCTATACGGTTCAAATATGGGTACCGCTGAAGGAACGGCGC
GTGATTTAGCAGATATTGCAATGAGCAAAGGATTTGCACCGCAGGTCGCAACGCTTGATTCACAC
GCCGGAAATCTTCCGCGCGAAGGAGCTGTATTAATTGTAACGGCGTCTTATAACGGTCATCCGCC
TGATAACGCAAAGCAATTTGTCGACTGGTTAGACCAAGCGTCTGCTGATGAAGTAAAAGGCGTTC
GCTACTCCGTATTTGGATGCGGCGATAAAAACTGGGCTACTACGTATCAAAAAGTGCCTGCTTTT
ATCGATGAAACGCTTGCCGCTAAAGGGGCAGAAAACATCGCTGACCGCGGTGAAGCAGATGCAAG
CGACGACTTTGAAGGCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAGCAGCCTACT
TTAACCTCGACATTGAAAACAGTGAAGATAATAAATCTACTCTTTCACTTCAATTTGTCGACAGC

GCCGCGGATATGCCGCTTGCGAAAATGCACGGTGCGTTTTCAACGCTCGAGCACCACCACCACCA
CCACTGA

MTIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKEACDE
SRFDKELSQGLKFLRDFLGDGL**P**TSWTHEKNWKKAHNILLPSFSQQAMKGYHASMVDIAVQLVQK
WERLNADEHIEVSEDMTRLTLDTIGLCGFNYRLNSFYRDQPHPFIISLVRALDEVMNKLQRANPD
DPAYDENKRQFQEDIKVMNDLVDKIIADRKARGEQSDDLLTQMLNGKDPETGEPLDDGNIRYQII
TFLY**S**GVEGTSGLLSFALYFLVKNPHVLQKVAEEAARVLVDPVPSYKQVKQLKYVGMVLNEALRL
WP**P**VPYF**A**LYAKEDTVLGGEYPLEKGDEVMVLIPQLHRDKTVWGDDVEEFRPERFENPSAIPQHA
FKPFGNGQRASLGQQFALHEATLVLGMMLKHFDFEDHTNYELDIKEL**L**TLKPKGFVVKAKSKKIP
LGGIPSPSTEQSAKKVRKKAENAHNTPLLVLYGSNMGTAEGTARDLADIAMSKGFAPQVATLDSH
AGNLPREGAVLIVTASYNGHPPDNAKQFVDWLDQASADEVKGVRYSVFGCGDKNWATTYQKVPAF
IDETLAAKGAENIADRGEADASDDFEGTYEEWREHMWSDVAAYFNLDIENSEDNKSTLSLQFVDS
AADMPLAKMHGAFSTLEHHHHHH

Nucleotide and amino acid sequences of P411-L8:

ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCGTTATTAAA
CACAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGAGAAATCTTTAAATTCG
AGGCGCCTGGTCGTGTAACGCGCTACTTATCAAGTCAGCGTCTAATTAAAGAAGCATGCGATGAA
TCACGCTTTGATAAAGAGTTAAGTCAAGGTCTGAAATTTCTGCGTGATTTTCTTGGAGACGGGTT
A**CCG**ACAAGCTGGACGCATGAAAAAAATTGGAAAAAAGCGCATAATATCTTACTTCCAAGCTTTA
GTCAGCAGGCAATGAAAGGCTATCATGCGAGTATGGTCGATATCGCCGTGCAGCTTGTTCAAAAG
TGGGAGCGTCTAAATGCAGATGAGCATATTGAAGTATCGGAAGACATGACACGTTTAACGCTTGA
TACAATTGGTCTTTGCGGCTTTAACTATCGCCTTAACAGCTTTTACCGAGATCAGCCTCATCCAT
TTATTATAAGTCTGGTCCGTGCACTGGATGAAGTAATGAACAAGCTGCAGCGAGCAAATCCAGAC
GACCCAGCTTATGATGAAAACAAGCGCCAGTTTCAAGAAGATATCAAGGTGATGAACGACCTAGT
AGATAAAATTATTGCAGATCGCAAAGCAAGGGGTGAACAAAGCGATGATTTATTAACGCAGATGC
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ACATTCTTATAT**AGT**GGAGTT**GAT**GGTACAAGTGGTCTTTTATCATTTGCGCTGTATTTCTTAGT
GAAAAATCCACATGTATTACAAAAAGTAGCAGAAGAAGCAGCACGAGTTCTAGTAGATCCTGTTC
CAAGCTACAAACAAGTCAAACAGCTTAAATATGTCGGCATGGTCTTAAACGAAGCGCTGCGCTTA
TGGCCA**CCG**GTTCCTTATTTT**GCG**CTATATGCAAAAGAAGATACGGTGCTTGGAGGAGAATATCC
TTTAGAAAAAGGCGACGAAGTAATGGTTCTGATTCCTCAGCTTCACCGTGATAAAACAGTTTGGG
GAGACGATGTGGAGGAGTTCCGTCCAGAGCGTTTTGAAAATCCAAGTGCGATTCCGCAGCATGCG
TTTAAACCGTTTGGAAACGGTCAGCGTGCGTCTCTGGGTCAGCAGTTCGCTCTTCATGAAGCAAC
GCTGGTACTTGGTATGATGCTAAAACACTTTGACTTTGAAGATCATACAAACTACGAGCTCGATA
TTAAAGAACTG**CTT**ACGTTAAAACCTAAAGGCTTTGTGGTAAAAGCAAAATCGAAAAAAATTCCG
CTTGGCGGTATTCCTTCACCTAGCACTGAACAGTCTGCTAAAAAAGTACGCAAAAAGGCAGAAAA
CGCTCATAATACGCCGCTGCTTGTGCTATACGGTTCAAATATGGGTACCGCTGAAGGAACGGCGC
GTGATTTAGCAGATATTGCAATGAGCAAAGGATTTGCACCGCAGGTCGCAACGCTTGATTCACAC
GCCGGAAATCTTCCGCGCGAAGGAGCTGTATTAATTGTAACGGCGTCTTATAACGGTCATCCGCC
TGATAACGCAAAGCAATTTGTCGACTGGTTAGACCAAGCGTCTGCTGATGAAGTAAAAGGCGTTC
GCTACTCCGTATTTGGATGCGGCGATAAAAACTGGGCTACTACGTATCAAAAAGTGCCTGCTTTT
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CGACGACTTTGAAGGCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAGCAGCCTACT
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GCCGCGGATATGCCGCTTGCGAAAATGCACGGTGCGTTTTCAACGCTCGAGCACCACCACCACCA
CCACTGA

MTIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKEACDE
SRFDKELSQGLKFLRDFLGDGL**P**TSWTHEKNWKKAHNILLPSFSQQAMKGYHASMVDIAVQLVQK

WERLNADEHIEVSEDMTRLTLDTIGLCGFNYRLNSFYRDQPHPFIISLVRALDEVMNKLQRANPD
DPAYDENKRQFQEDIKVMNDLVDKIIADRKARGEQSDDLLTQMLNGKDPETGEPLDDGNIRYQII
TFLY**S**GV**D**GTSGLLSFALYFLVKNPHVLQKVAEEAARVLVDPVPSYKQVKQLKYVGMVLNEALRL
WP**P**VPYF**A**LYAKEDTVLGGEYPLEKGDEVMVLIPQLHRDKTVWGDDVEEFRPERFENPSAIPQHA
FKPFGNGQRASLGQQFALHEATLVLGMMLKHFDFEDHTNYELDIKEL**L**TLKPKGFVVKAKSKKIP
LGGIPSPSTEQSAKKVRKKAENAHNTPLLVLYGSNMGTAEGTARDLADIAMSKGFAPQVATLDSH
AGNLPREGAVLIVTASYNGHPPDNAKQFVDWLDQASADEVKGVRYSVFGCGDKNWATTYQKVPAF
IDETLAAKGAENIADRGEADASDDFEGTYEEWREHMWSDVAAYFNLDIENSEDNKSTLSLQFVDS
AADMPLAKMHGAFSTLEHHHHHH

Nucleotide and amino acid sequences of P411-L9:

ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCGTTATTAAA
CACAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGAGAAATCTTTAAATTCG
AGGCGCCTGGTCGTGTAACGCGCTACTTATCAAGTCAGCGTCTAATTAAAGAAGCATGCGATGAA
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A**CCG**ACAAGCTGGACGCATGAAAAAAATTGGAAAAAAGCGCATAATATCTTACTTCCAAGCTTTA
GTCAGCAGGCAATGAAAGGCTATCATGCGAGTATGGTCGATATCGCCGTGCAGCTTGTTCAAAAG
TGGGAGCGTCTAAATGCAGATGAGCATATTGAAGTATCGGAAGACATGACACGTTTAACGCTTGA
TACAATTGGTCTTTGCGGCTTTAACTATCGCCTTAACAGCTTTTACCGAGATCAGCCTCATCCAT
TTATTATAAGTCTGGTCCGTGCACTGGATGAAGTAATGAACAAGCTGCAGCGAGCAAATCCAGAC
GACCCAGCTTATGATGAAAACAAGCGCCAGTTTCAAGAAGATATCAAGGTGATGAACGACCTAGT
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TAAACGGAAAAGATCCAGAAACGGGTGAGCCGCTTGATGACGGGAACATTCGCTATCAAATTATT
ACATTCTTATAT**AGT**GGAGTT**GAT**GGTACAAGTGGTCTTTTATCATTTGCGCTGTATTTCTTAGT
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GAGACGATGTGGAGGAGTTCCGTCCAGAGCGTTTTGAAAATCCAAGTGCGATTCCGCAGCATGCG
TTTAAACCGTTTGGAAACGGTCAGCGTGCGTCTCTGGGTCAGCAGTTCGCTCTTCATGAAGCAAC
GCTGGTACTTGGTATGATGCTAAAACACTTTGACTTTGAAGATCATACAAACTACGAGCTCGATA
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CTTGGCGGTATTCCTTCACCTAGCACTGAACAGTCTGCTAAAAAAGTACGCAAAAAGGCAGAAAA
CGCTCATAATACGCCGCTGCTTGTGCTATACGGTTCAAATATGGGTACCGCTGAAGGAACGGCGC
GTGATTTAGCAGATATTGCAATGAGCAAAGGATTTGCACCGCAGGTCGCAACGCTTGATTCACAC
GCCGGAAATCTTCCGCGCGAAGGAGCTGTATTAATTGTAACGGCGTCTTATAACGGTCATCCGCC
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CGACGACTTTGAAGGCACATATGAAGAATGGCGTGAACATATGTGGAGTGACGTAGCAGCCTACT
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GCCGCGGATATGCCGCTTGCGAAAATGCACGGTGCGTTTTCAACGCTCGAGCACCACCACCACCA
CCACTGA

MTIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKEACDE
SRFDKELSQGLKFLRDFLGDGL**P**TSWTHEKNWKKAHNILLPSFSQQAMKGYHASMVDIAVQLVQK
WERLNADEHIEVSEDMTRLTLDTIGLCGFNYRLNSFYRDQPHPFIISLVRALDEVMNKLQRANPD
DPAYDENKRQFQEDIKVMNDLVDKIIADRKARGEQSDDLLTQMLNGKDPETGEPLDDGNIRYQII
TFLY**S**GV**D**GTSGLLSFALYFLVKNPHVLQKVAEEAARVLVDPVPSYKQVKQLKYVGMVLNEALRL
WP**PL**PYF**A**LYAKEDTVLGGEYPLEKGDEVMVLIPQLHRDKTVWGDDVEEFRPERFENPSAIPQHA
FKPFGNGQRASLGQQFALHEATLVLGMMLKHFDFEDHTNYELDIKEL**L**TLKPKGFVVKAKSKKIP

LGGIPSPSTEQSAKKVRKKAENAHNTPLLVLYGSNMGTAEGTARDLADIAMSKGFAPQVATLDSH
AGNLPREGAVLIVTASYNGHPPDNAKQFVDWLDQASADEVKGVRYSVFGCGDKNWATTYQKVPAF
IDETLAAKGAENIADRGEADASDDFEGTYEEWREHMWSDVAAYFNLDIENSEDNKSTLSLQFVDS
AADMPLAKMHGAFSTLEHHHHHH

Nucleotide and amino acid sequences of P411-L10:

ATGACAATTAAAGAAATGCCTCAGCCAAAAACGTTTGGAGAGCTTAAAAATTTACCGTTATTAAA
CACAGATAAACCGGTTCAAGCTTTGATGAAAATTGCGGATGAATTAGGAGAAATCTTTAAATTCG
AGGCGCCTGGTCGTGTAACGCGCTACTTATCAAGTCAGCGTCTAATTAAAGAAGCATGCGATGAA
TCACGCTTTGATAAAGAGTTAAGTCAAGGTCTGAAATTTCTGCGTGATTTTCTTGGAGACGGGTT
A**CCG**ACAAGCTGGACGCATGAAAAAAATTGGAAAAAAGCGCATAATATCTTACTTCCAAGCTTTA
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AGATAAAATTATTGCAGATCGCAAAGCAAGGGGTGAACAAAGCGATGATTTATTAACGCAGATGC
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CCACTGA

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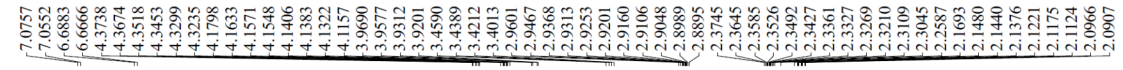
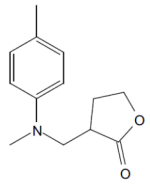
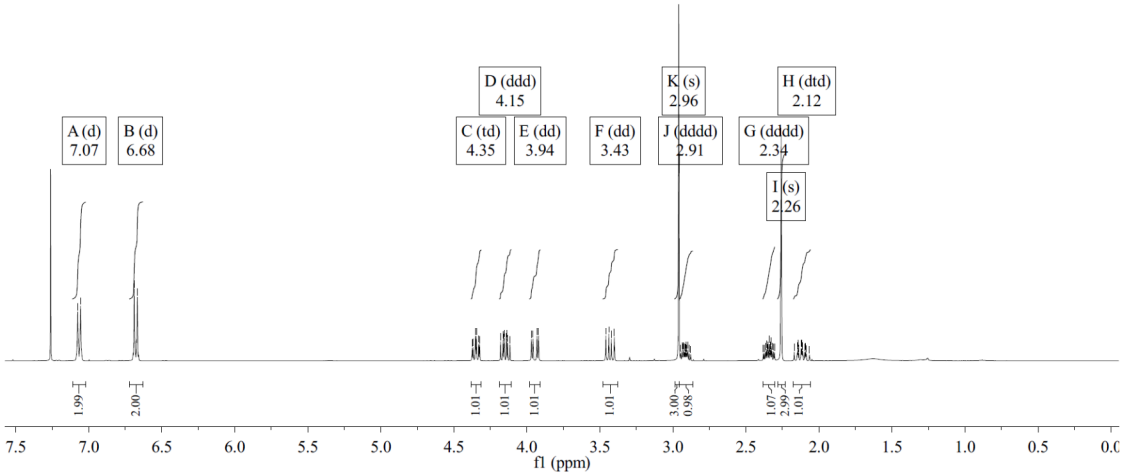
WERLNADEHIEVSEDMTRLTLDTIGLCGFNYRLNSFYRDQPHPFIISLVRALDEVMNKLQRANPD
DPAYDENKRQFQEDIKVMNDLVDKIIADRKARGEQSDDLLTQMLNGKDPETGEPLDDGNIRYQII
TFLY**S**GV**D**GTSGLLSFALYFLVKNPHVLQKVAEEAARVLVDPVPSYKQVKQLKYVGMVLNEALRL
WP**PR**PYF**A**LYAKEDTVLGGEYPLEKGDEVMVLIPQLHRDKTVWGDDVEEFRPERFENPSAIPQHA
FKPFGNGQRASLGQQFALHEATLVLGMMLKHFDFEDHTNYELDIKEL**L**TLKPKGFVVKAKSKKIP
LGGIPSPSTEQSAKKVRKKAENAHNTPLLVLYGSNMGTAEGTARDLADIAMSKGFAPQVATLDSH
AGNLPREGAVLIVTASYNGHPPDNAKQFVDWLDQASADEVKGVRYSVFGCGDKNWATTYQKVPAF
IDETLAAKGAENIADRGEADASDDFEGTYEEWREHMWSDVAAYFNLDIENSEDNKSTLSLQFVDS
AADMPLAKMHGAFSTLEHHHHHH

VII. NMR Spectra

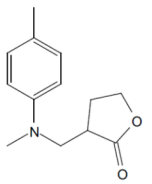


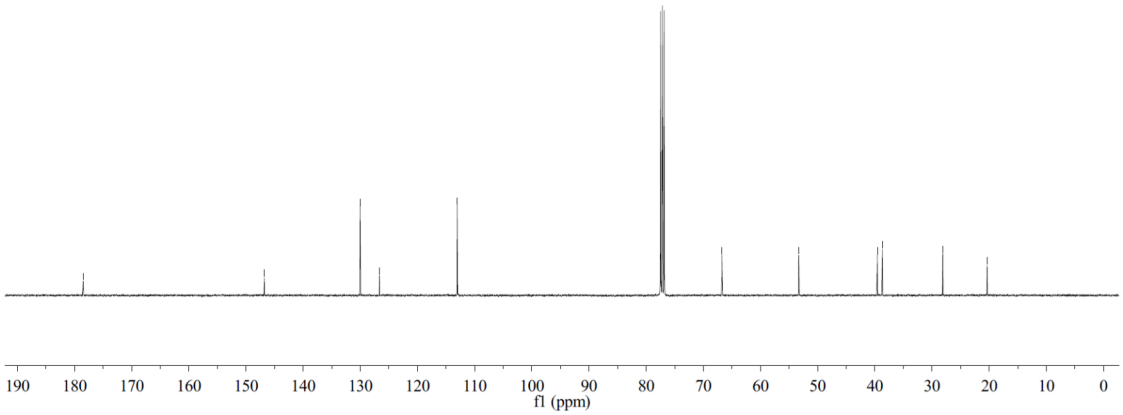
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.07 (d, *J* = 8.2 Hz, 2H), 6.68 (d, *J* = 8.7 Hz, 2H), 4.35 (td, *J* = 8.8, 2.6 Hz, 1H), 4.15 (ddd, *J* = 10.0, 9.1, 6.6 Hz, 1H), 3.94 (dd, *J* = 15.1, 4.5 Hz, 1H), 3.43 (dd, *J* = 15.1, 8.0 Hz, 1H), 2.96 (s, 3H), 2.91 (dddd, *J* = 10.6, 8.6, 8.0, 4.5 Hz, 1H), 2.34 (dddd, *J* = 12.7, 9.0, 6.6, 2.6 Hz, 1H), 2.26 (s, 3H), 2.12 (dtd, *J* = 12.7, 10.2, 8.6 Hz, 1H).

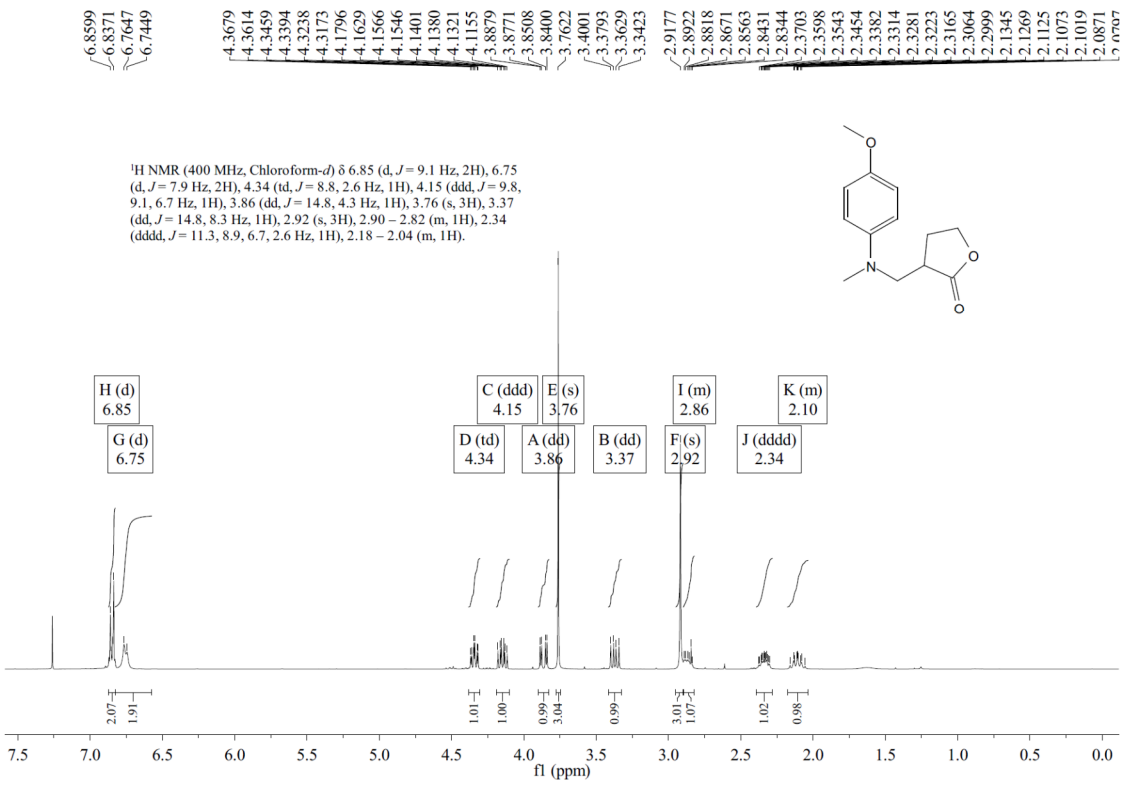


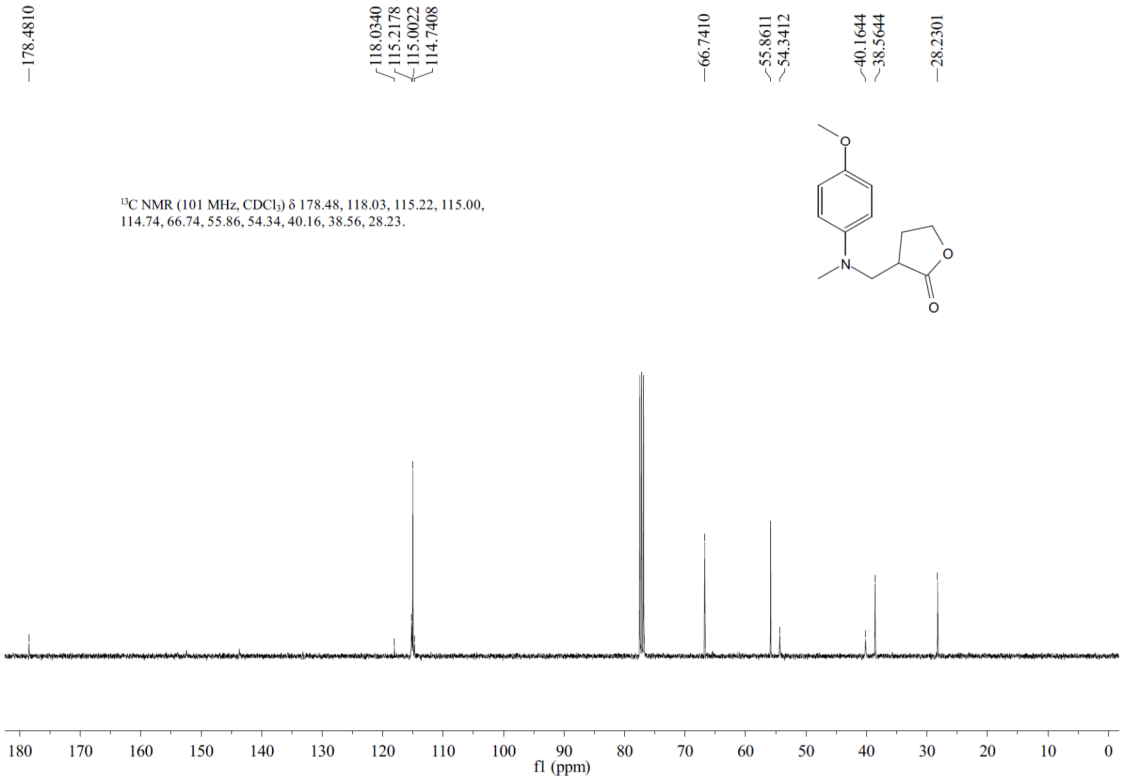


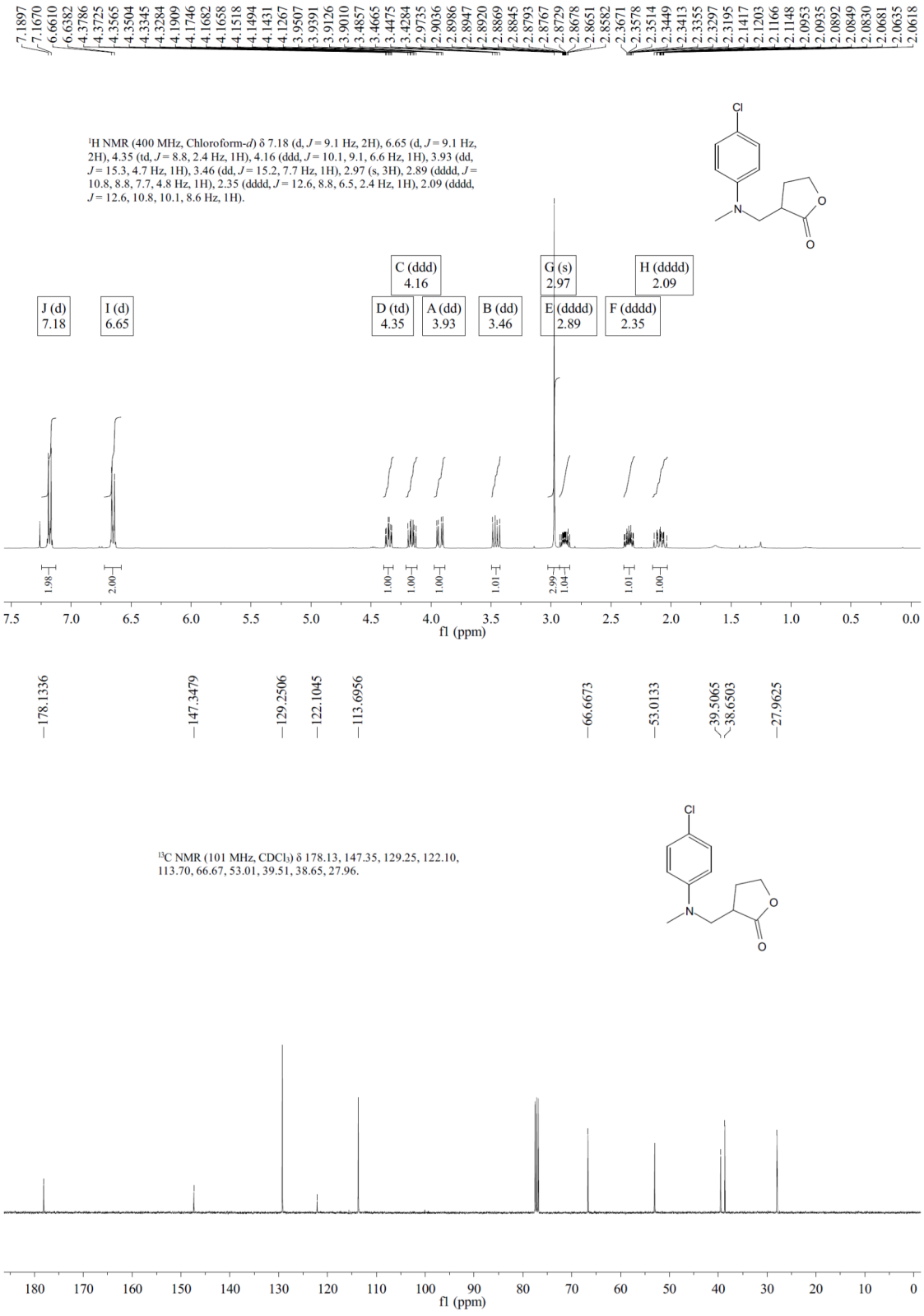
^{13}C NMR (101 MHz, $CDCl_3$) δ 178.48, 146.77, 130.03, 126.65, 113.06, 66.74, 53.31, 39.56, 38.67, 28.10, 20.36.





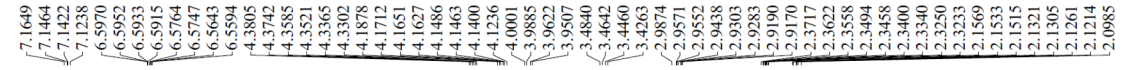

6.8599
6.8371
6.7647
6.7449
4.3679
4.3614
4.3459
4.3394
4.3238
4.3173
4.1796
4.1629
4.1566
4.1546
4.1401
4.1380
4.1321
4.1155
3.8879
3.8771
3.8508
3.8400
3.7622
3.4001
3.3793
3.3629
3.3423
2.9177
2.8922
2.8818
2.8671
2.8563
2.8431
2.8344
2.3703
2.3598
2.3543
2.3454
2.3382
2.3314
2.3281
2.3223
2.3165
2.3064
2.2999
2.1345
2.1269
2.1125
2.1073
2.1019
2.0871
1H NMR (400 MHz, Chloroform-d) δ 6.85 (d, J = 9.1 Hz, 2H), 6.75 (d, J = 7.9 Hz, 2H), 4.34 (td, J = 8.8, 2.6 Hz, 1H), 4.15 (ddd, J = 9.8, 9.1, 6.7 Hz, 1H), 3.86 (dd, J = 14.8, 4.3 Hz, 1H), 3.76 (s, 3H), 3.37 (dd, J = 14.8, 8.3 Hz, 1H), 2.92 (s, 3H), 2.90 – 2.82 (m, 1H), 2.34 (dddd, J = 11.3, 8.9, 6.7, 2.6 Hz, 1H), 2.18 – 2.04 (m, 1H).
H (d) 6.85
G (d) 6.75
C (ddd) 4.15
E (s) 3.76
D (td) 4.34
A (dd) 3.86
B (dd) 3.37
I (m) 2.86
F (s) 2.92
K (m) 2.10
J (dddd) 2.34
2.07
1.91
1.01
1.00
0.99
3.04
0.99
3.01
1.07
1.02
0.98
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


178.4810
118.0340
115.2178
115.0022
114.7408
66.7410
55.8611
54.3412
40.1644
38.5644
28.2301
13C NMR (101 MHz, CDCl3) δ 178.48, 118.03, 115.22, 115.00, 114.74, 66.74, 55.86, 54.34, 40.16, 38.56, 28.23.
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

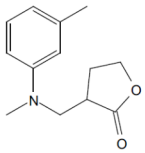


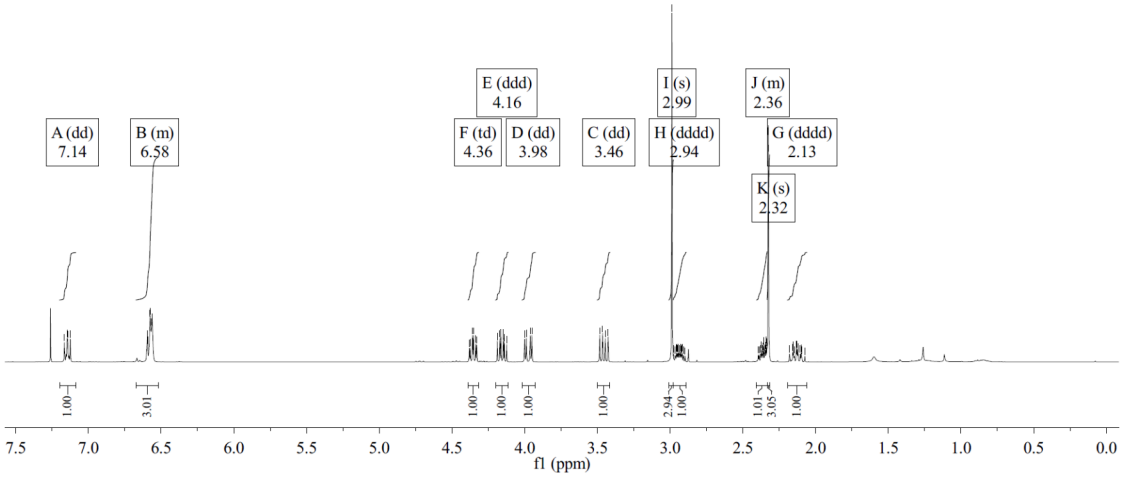
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.18 (d, *J* = 9.1 Hz, 2H), 6.65 (d, *J* = 9.1 Hz, 2H), 4.35 (td, *J* = 8.8, 2.4 Hz, 1H), 4.16 (ddd, *J* = 10.1, 9.1, 6.6 Hz, 1H), 3.93 (dd, *J* = 15.3, 4.7 Hz, 1H), 3.46 (dd, *J* = 15.2, 7.7 Hz, 1H), 2.97 (s, 3H), 2.89 (dddd, *J* = 10.8, 8.8, 7.7, 4.8 Hz, 1H), 2.35 (dddd, *J* = 12.6, 8.8, 6.5, 2.4 Hz, 1H), 2.09 (dddd, *J* = 12.6, 10.8, 10.1, 8.6 Hz, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 178.13, 147.35, 129.25, 122.10, 113.70, 66.67, 53.01, 39.51, 38.65, 27.96.

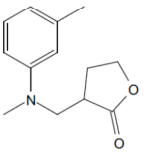


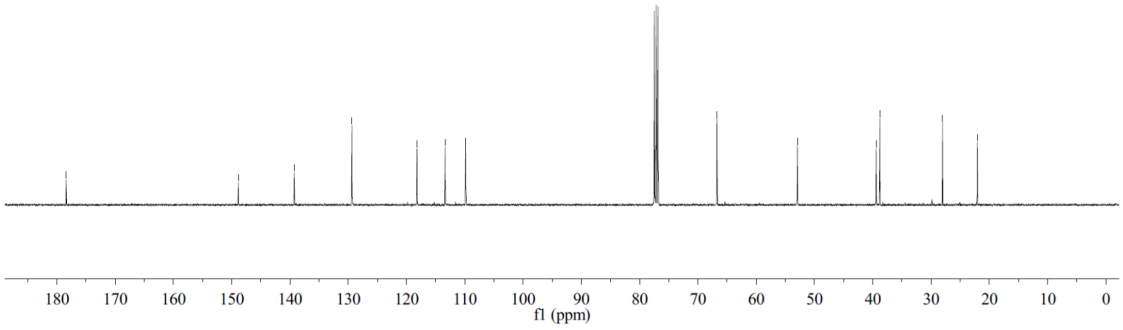
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.14 (dd, *J* = 9.1, 7.4 Hz, 1H), 6.67 – 6.52 (m, 3H), 4.36 (td, *J* = 8.8, 2.5 Hz, 1H), 4.16 (ddd, *J* = 10.0, 9.1, 6.6 Hz, 1H), 3.98 (dd, *J* = 15.2, 4.6 Hz, 1H), 3.46 (dd, *J* = 15.2, 7.9 Hz, 1H), 2.99 (s, 3H), 2.94 (dddd, *J* = 10.7, 8.8, 8.0, 4.6 Hz, 1H), 2.41 – 2.33 (m, 1H), 2.32 (s, 3H), 2.13 (dddd, *J* = 12.7, 10.7, 10.0, 8.5 Hz, 1H).

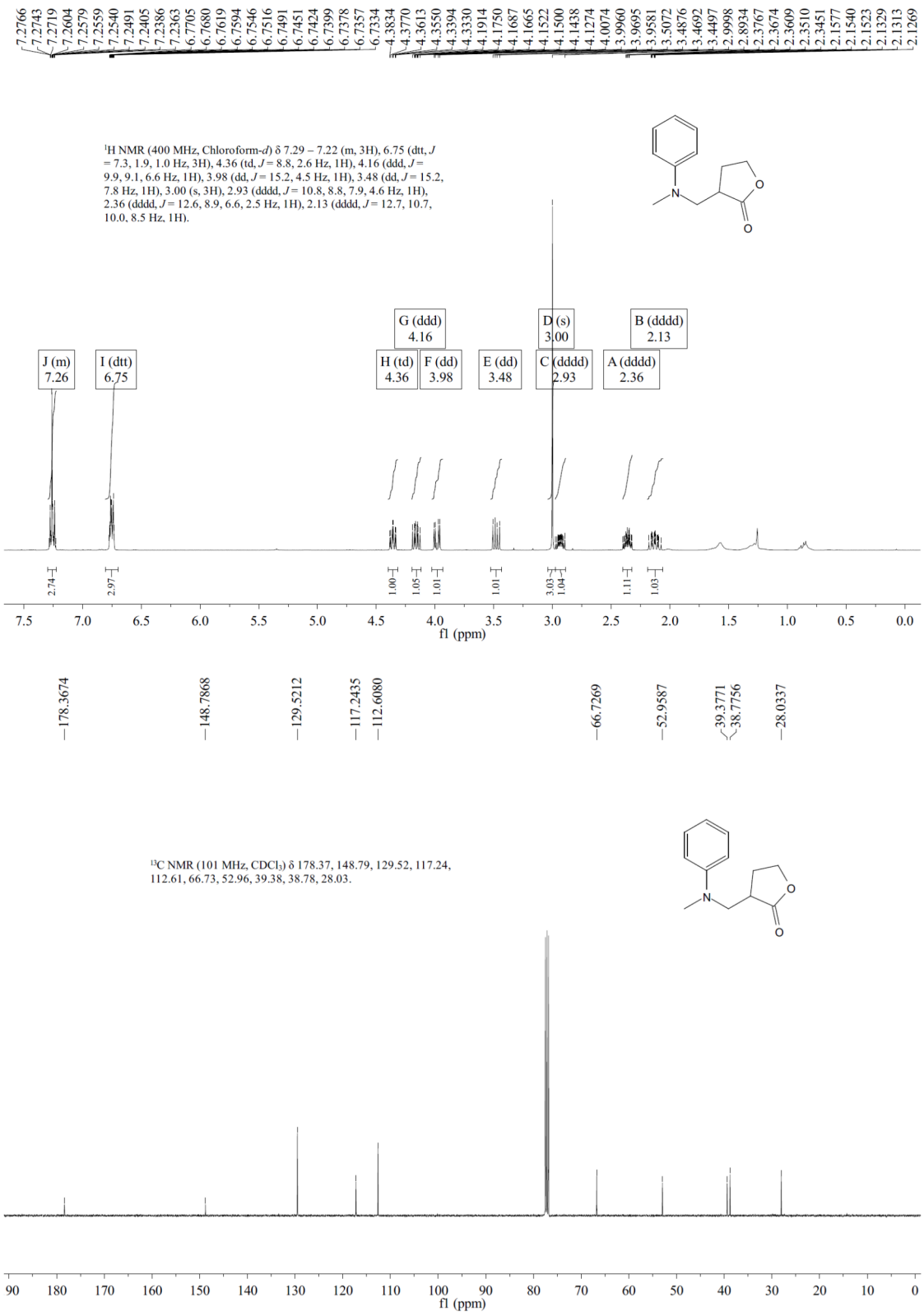


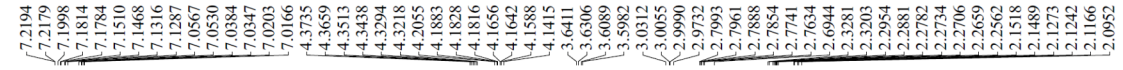


^{13}C NMR (101 MHz, $CDCl_3$) δ 178.40, 148.86, 139.25, 129.35, 118.19, 113.36, 109.84, 66.72, 52.94, 39.42, 38.77, 28.02, 22.07.

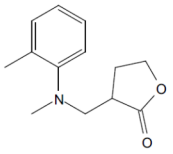


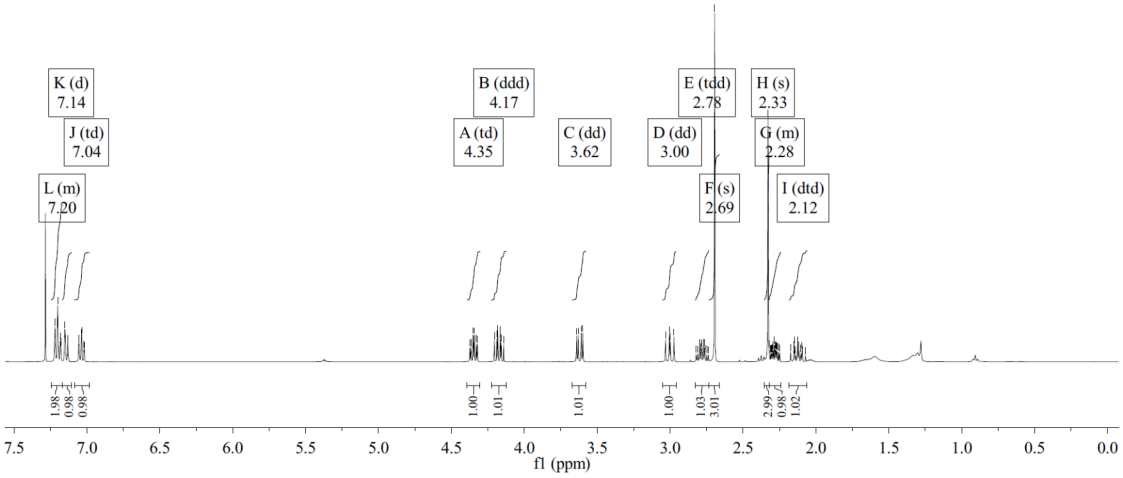



7.2766 7.2743 7.2719 7.2604 7.2579 7.2559 7.2540 7.2491 7.2405 7.2386 7.2363 7.7705 6.7680 6.7619 6.7594 6.7546 6.7516 6.7491 6.7451 6.7424 6.7399 6.7378 6.7357 6.7334
4.3834 4.3770 4.3613 4.3550 4.3394 4.3330 4.1914 4.1750 4.1687 4.1665 4.1522 4.1500 4.1438 4.1274 4.0074 3.9960 3.9695 3.9581 3.5072 3.4876 3.4692 3.4497 2.9998 2.8934 2.3767 2.3674 2.3609 2.3510 2.3451 2.1577 2.1540 2.1523 2.1329 2.1313 2.1269
1H NMR (400 MHz, Chloroform-d) δ 7.29 – 7.22 (m, 3H), 6.75 (dtt, J = 7.3, 1.9, 1.0 Hz, 3H), 4.36 (td, J = 8.8, 2.6 Hz, 1H), 4.16 (ddd, J = 9.9, 9.1, 6.6 Hz, 1H), 3.98 (dd, J = 15.2, 4.5 Hz, 1H), 3.48 (dd, J = 15.2, 7.8 Hz, 1H), 3.00 (s, 3H), 2.93 (dddd, J = 10.8, 8.8, 7.9, 4.6 Hz, 1H), 2.36 (dddd, J = 12.6, 8.9, 6.6, 2.5 Hz, 1H), 2.13 (dddd, J = 12.7, 10.7, 10.0, 8.5 Hz, 1H).
N
O
O
J (m) 7.26
I (dtt) 6.75
G (ddd) 4.16
H (td) 4.36
F (dd) 3.98
E (dd) 3.48
D (s) 3.00
C (dddd) 2.93
B (dddd) 2.13
A (dddd) 2.36
2.74
2.97
1.00
1.05
1.01
1.01
3.03
1.04
1.11
1.03
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)
178.3674
148.7868
129.5212
117.2435
112.6080
66.7269
52.9587
39.3771
38.7756
28.0337
13C NMR (101 MHz, CDCl3) δ 178.37, 148.79, 129.52, 117.24, 112.61, 66.73, 52.96, 39.38, 38.78, 28.03.
N
O
O
90 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

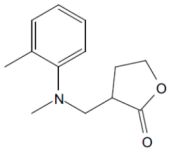


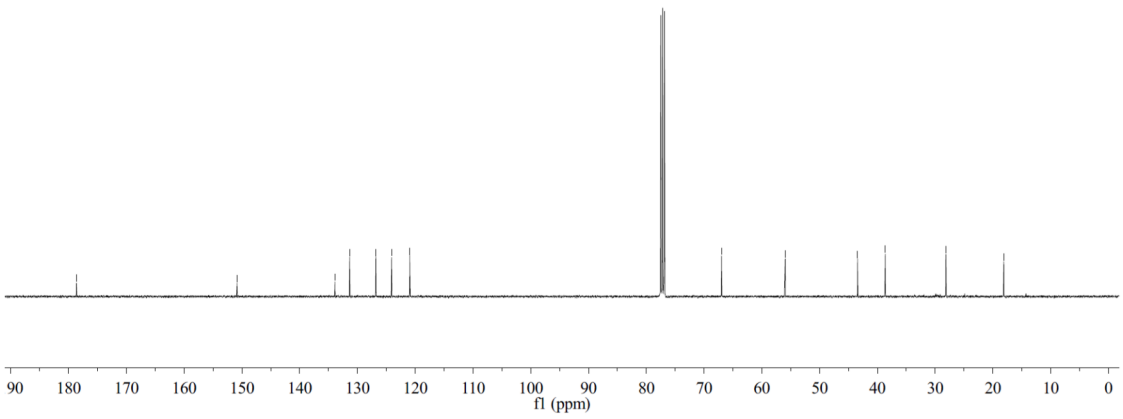
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.24 – 7.17 (m, 2H), 7.14 (d, *J* = 7.8 Hz, 1H), 7.04 (td, *J* = 7.3, 1.5 Hz, 1H), 4.35 (td, *J* = 8.8, 3.0 Hz, 1H), 4.17 (ddd, *J* = 9.6, 9.1, 6.9 Hz, 1H), 3.62 (dd, *J* = 12.9, 4.2 Hz, 1H), 3.00 (dd, *J* = 12.9, 10.3 Hz, 1H), 2.78 (tdd, *J* = 10.1, 8.8, 4.3 Hz, 1H), 2.69 (s, 3H), 2.33 (s, 3H), 2.32 – 2.24 (m, 1H), 2.12 (dtd, *J* = 12.9, 9.8, 8.6 Hz, 1H).



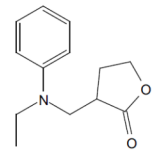


^{13}C NMR (101 MHz, $CDCl_3$) δ 178.66, 150.84, 133.89, 131.35, 126.82, 124.10, 120.95, 66.97, 55.95, 43.43, 38.63, 28.13, 18.12.

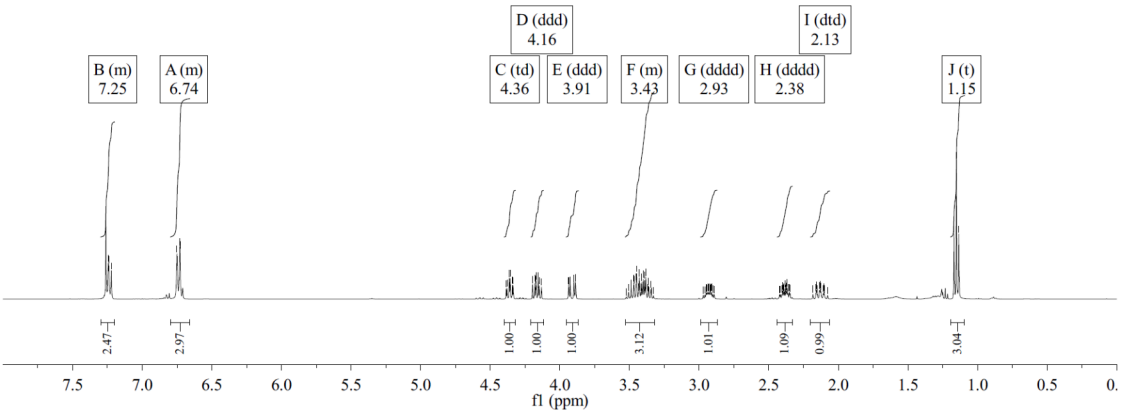




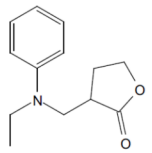
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.30 – 7.20 (m, 2H), 6.80 – 6.66 (m, 3H), 4.36 (td, *J* = 8.8, 2.5 Hz, 1H), 4.16 (ddd, *J* = 10.0, 9.0, 6.6 Hz, 1H), 3.91 (ddd, *J* = 15.1, 4.6, 0.7 Hz, 1H), 3.53 – 3.32 (m, 3H), 2.93 (dddd, *J* = 12.5, 7.9, 6.2, 2.8 Hz, 1H), 2.38 (dddd, *J* = 12.7, 8.9, 6.6, 2.5 Hz, 1H), 2.13 (dtd, *J* = 12.7, 10.3, 8.5 Hz, 1H), 1.15 (t, *J* = 7.0 Hz, 3H).



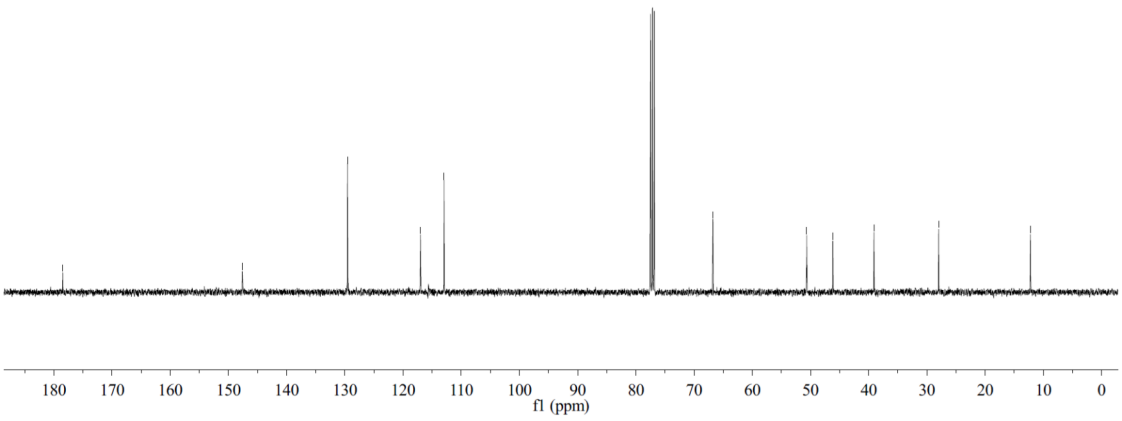
with L9

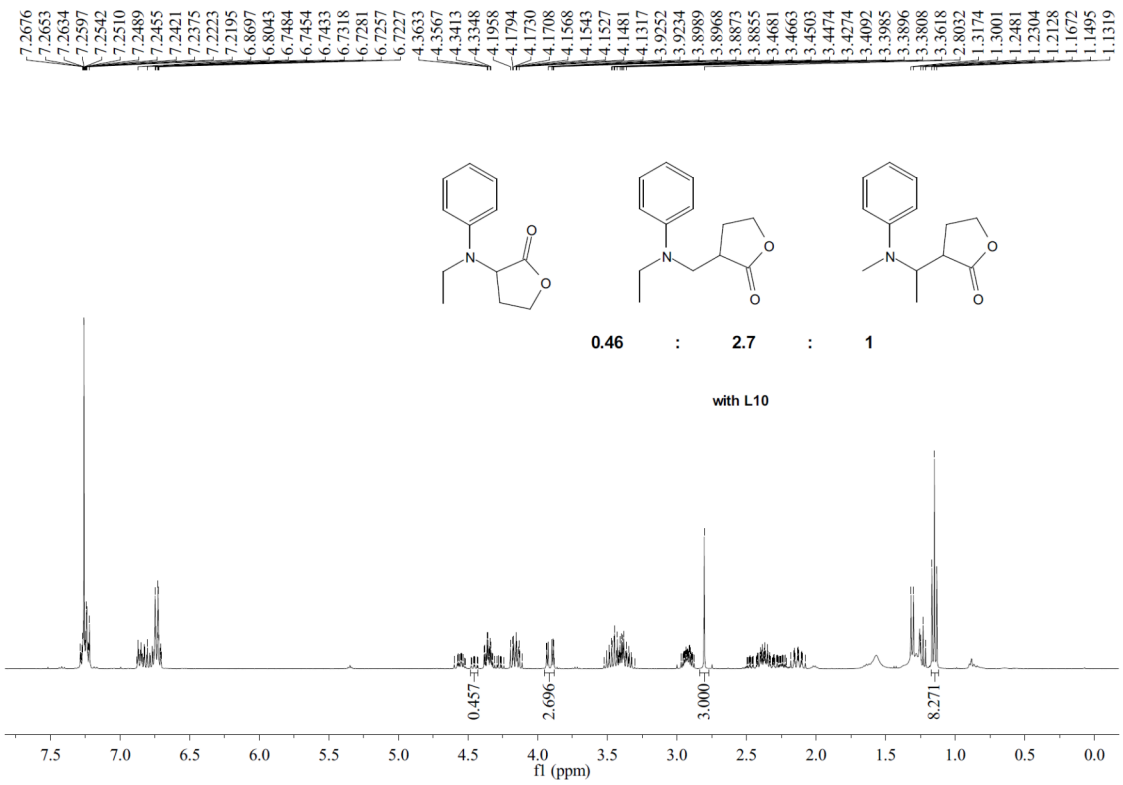


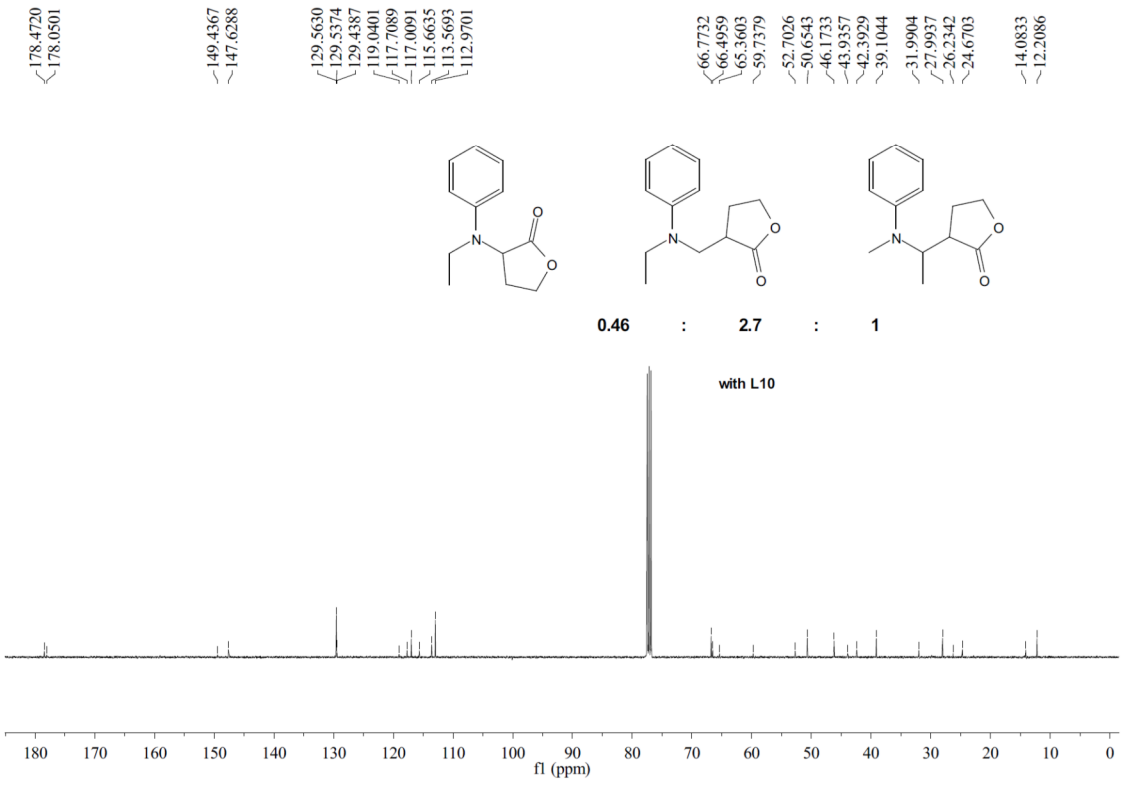
^{13}C NMR (101 MHz, $CDCl_3$) δ 178.46, 147.62, 129.55, 117.00, 112.96, 66.77, 50.65, 46.16, 39.09, 27.98, 12.20.

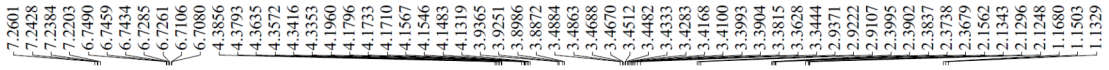


with L9

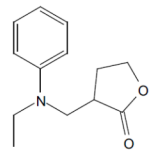


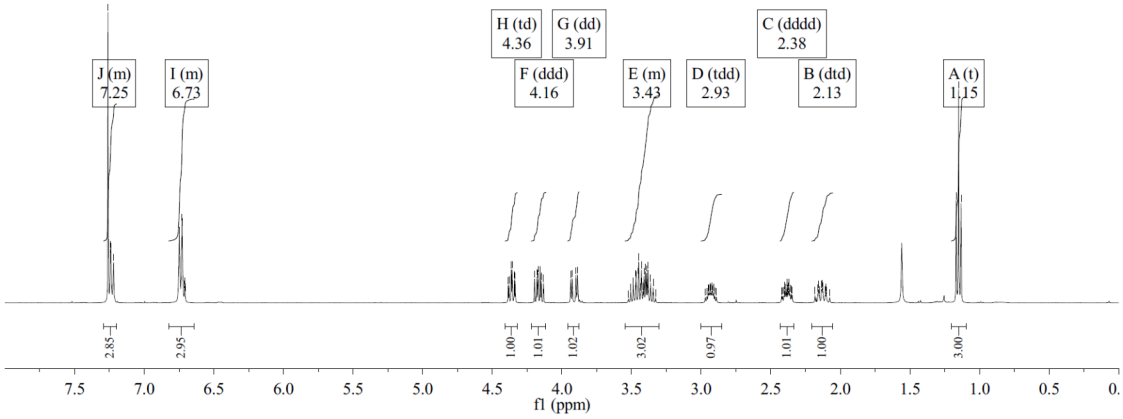

7.2676
7.2653
7.2634
7.2597
7.2542
7.2510
7.2489
7.2455
7.2421
7.2375
7.2223
7.2195
6.8697
6.8043
6.7484
6.7454
6.7433
6.7318
6.7281
6.7257
6.7227
4.3633
4.3567
4.3413
4.3348
4.1958
4.1794
4.1730
4.1708
4.1568
4.1543
4.1527
4.1481
4.1317
3.9252
3.9234
3.8989
3.8968
3.8873
3.8855
3.4681
3.4663
3.4503
3.4474
3.4274
3.4092
3.3985
3.3896
3.3808
3.3618
2.8032
1.3174
1.3001
1.2481
1.2304
1.2128
1.1672
1.1495
1.1319
0.46 : 2.7 : 1
with L10
0.457
2.696
3.000
8.271
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


178.4720
178.0501
149.4367
147.6288
129.5630
129.5374
129.4387
119.0401
117.7089
117.0091
115.6635
113.5693
112.9701
66.7732
66.4959
65.3603
59.7379
52.7026
50.6543
46.1733
43.9357
42.3929
39.1044
31.9904
27.9937
26.2342
24.6703
14.0833
12.2086
0.46 : 2.7 : 1
with L10
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

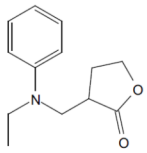


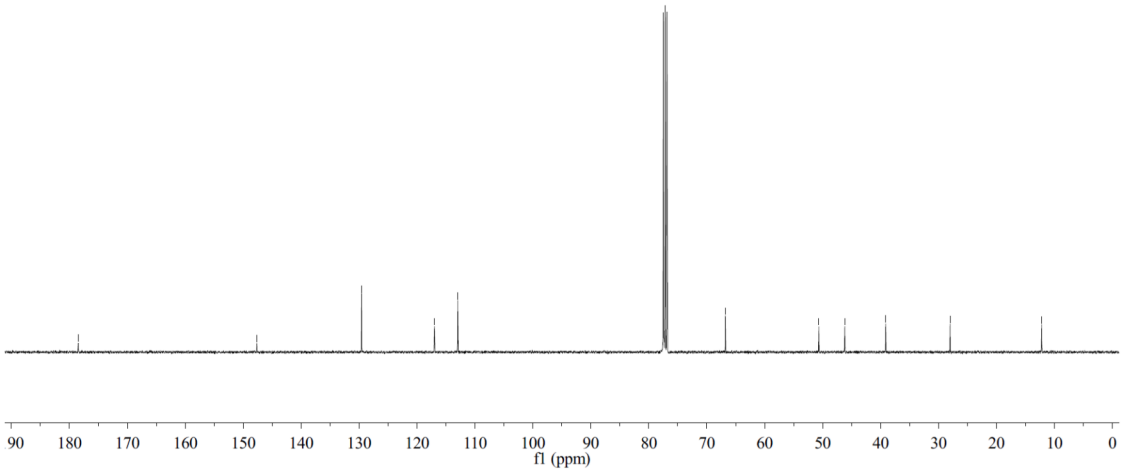
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.29 – 7.20 (m, 3H), 6.82 – 6.64 (m, 3H), 4.36 (td, *J* = 8.8, 2.5 Hz, 1H), 4.16 (ddd, *J* = 9.9, 9.1, 6.6 Hz, 1H), 3.91 (dd, *J* = 15.1, 4.6 Hz, 1H), 3.55 – 3.30 (m, 3H), 2.93 (tdd, *J* = 10.8, 8.3, 4.6 Hz, 1H), 2.38 (dddd, *J* = 12.5, 8.9, 6.6, 2.5 Hz, 1H), 2.13 (dtd, *J* = 12.6, 10.3, 8.7 Hz, 1H), 1.15 (t, *J* = 7.0 Hz, 3H).

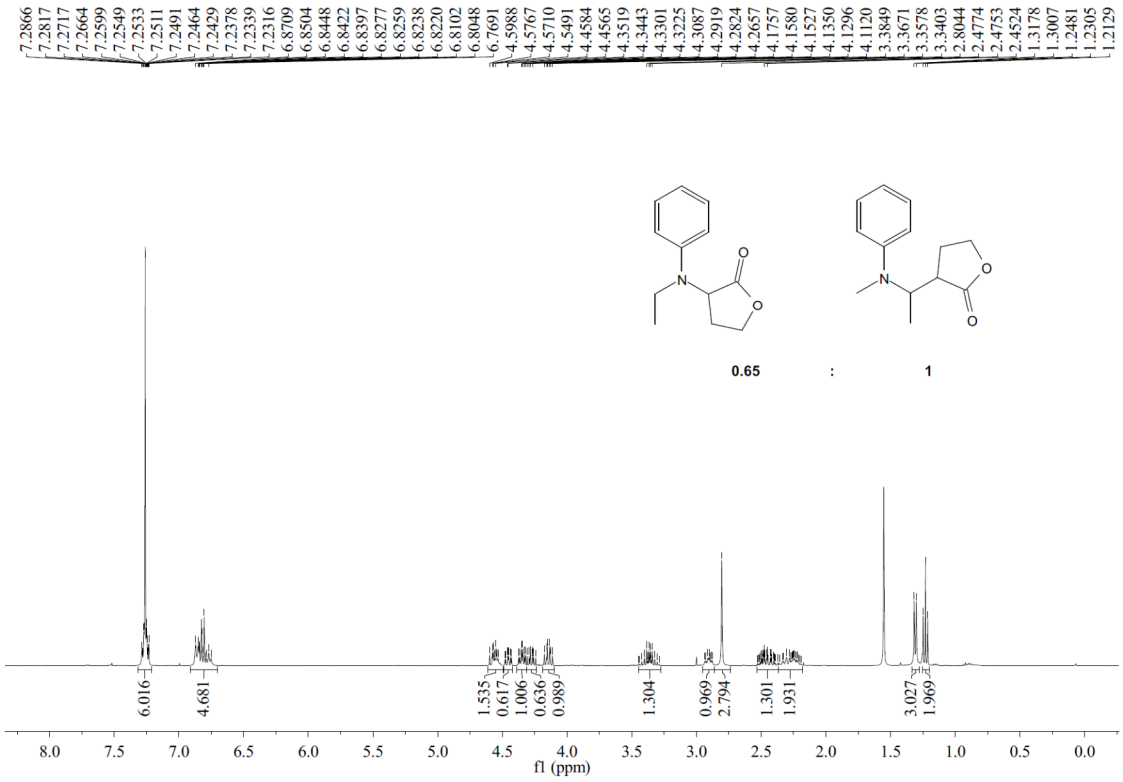


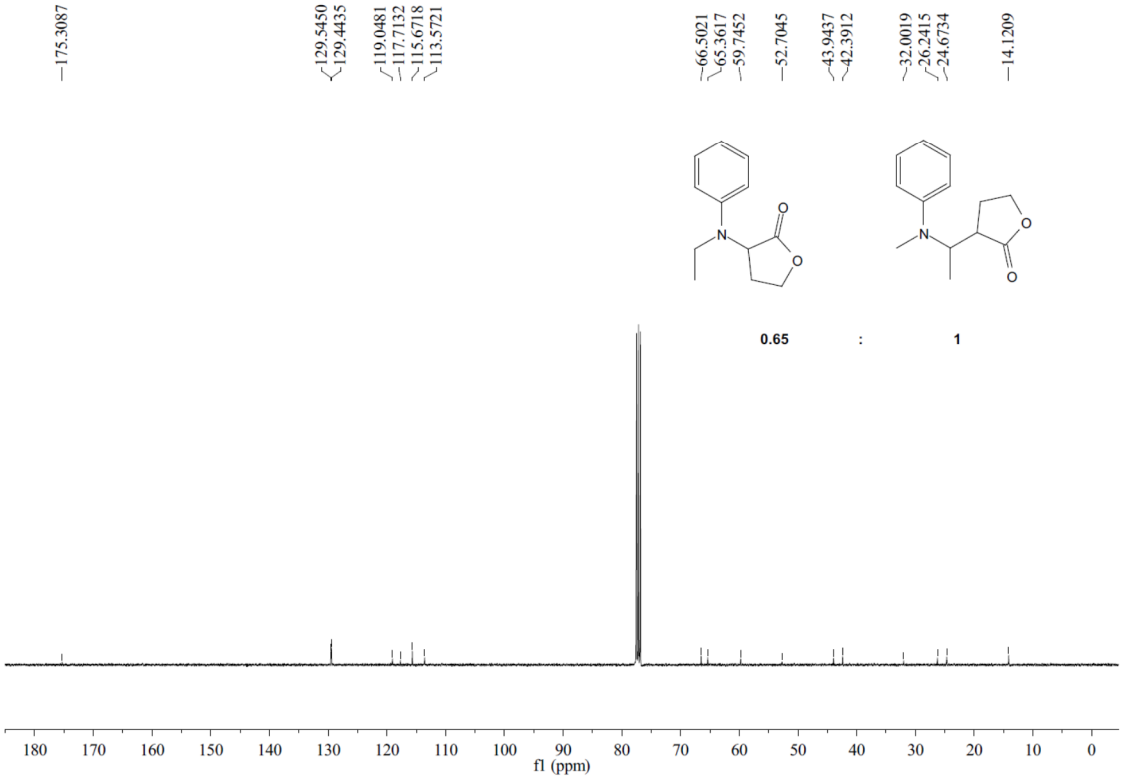


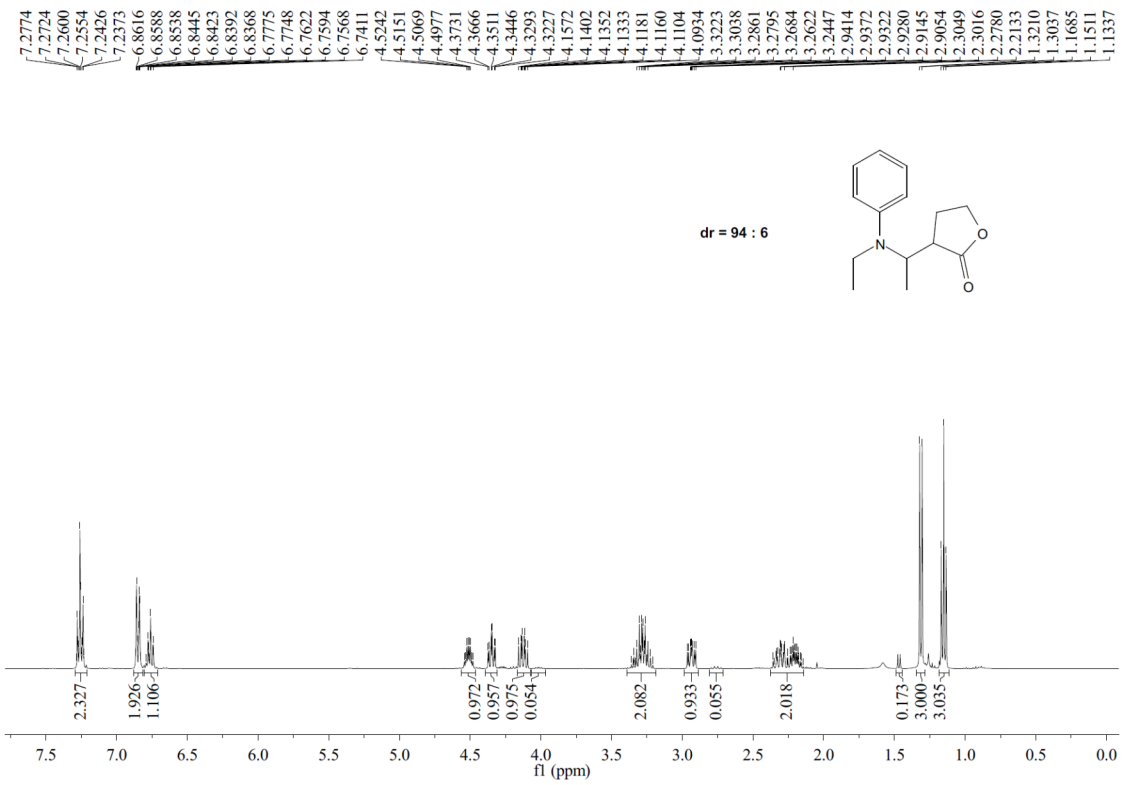
^{13}C NMR (101 MHz, $CDCl_3$) δ 178.47, 147.63, 129.56, 117.01, 112.97, 66.77, 50.65, 46.17, 39.10, 28.00, 12.21.

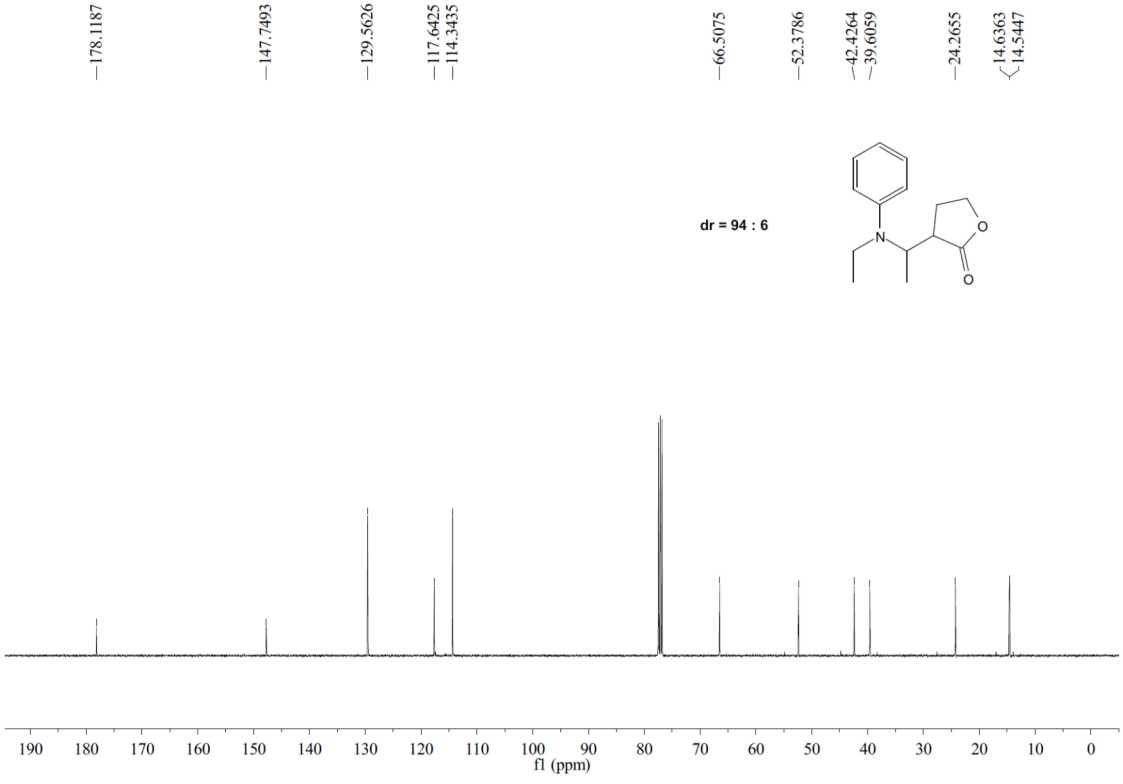


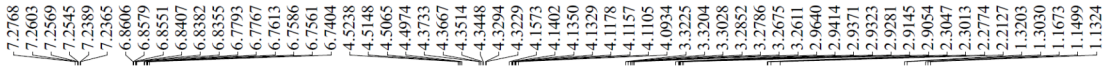



7.2866
7.2817
7.2717
7.2664
7.2599
7.2549
7.2533
7.2511
7.2491
7.2464
7.2429
7.2378
7.2339
7.2316
6.8709
6.8504
6.8448
6.8422
6.8397
6.8277
6.8259
6.8238
6.8220
6.8102
6.8048
6.7691
4.5988
4.5767
4.5710
4.5491
4.4584
4.4565
4.3519
4.3443
4.3301
4.3225
4.3087
4.2919
4.2824
4.2657
4.1757
4.1580
4.1527
4.1350
4.1296
4.1120
3.3849
3.3671
3.3578
3.3403
2.8044
2.4774
2.4753
2.4524
1.3178
1.3007
1.2481
1.2305
1.2129
0.65 : 1
6.016
4.681
1.535
0.617
1.006
0.636
0.989
1.304
0.969
2.794
1.301
1.931
3.027
1.969
f1 (ppm)

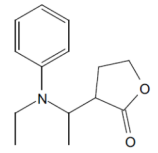

175.3087
129.5450
129.4435
119.0481
117.7132
115.6718
113.5721
66.5021
65.3617
59.7452
52.7045
43.9437
42.3912
32.0019
26.2415
24.6734
14.1209
0.65 : 1
f1 (ppm)


7.2774
7.2724
7.2600
7.2554
7.2426
7.2373
6.8616
6.8588
6.8538
6.8445
6.8423
6.8392
6.8368
6.7775
6.7748
6.7622
6.7594
6.7568
6.7411
4.5242
4.5151
4.5069
4.4977
4.3731
4.3666
4.3511
4.3446
4.3293
4.3227
4.1572
4.1402
4.1352
4.1333
4.1181
4.1160
4.1104
4.0934
3.3223
3.3038
3.2861
3.2795
3.2684
3.2622
3.2447
2.9414
2.9372
2.9322
2.9280
2.9145
2.9054
2.3049
2.3016
2.2780
2.2133
1.3210
1.3037
1.1685
1.1511
1.1337
dr = 94 : 6
2.327
1.926
1.106
0.972
0.957
0.975
0.054
2.082
0.933
0.055
2.018
0.173
3.000
3.035
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

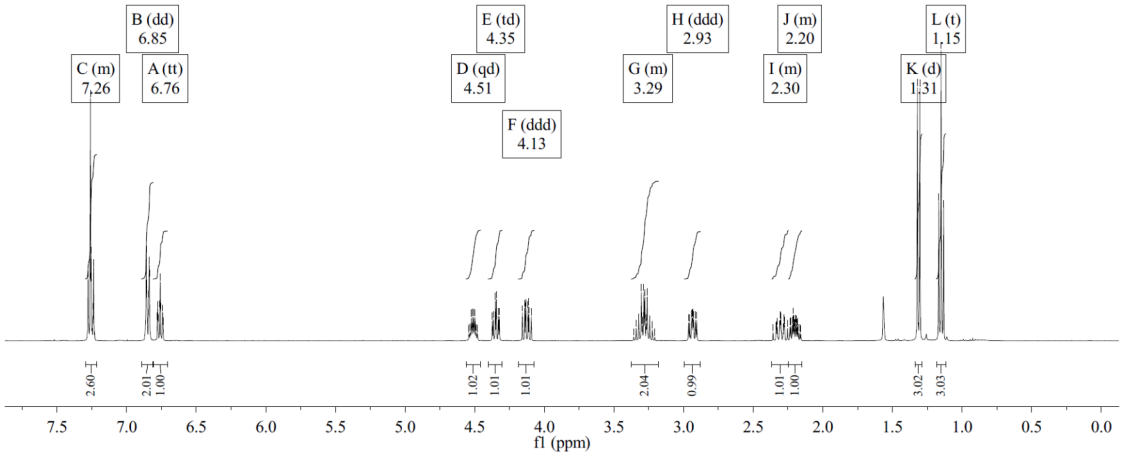

178.1187
147.7493
129.5626
117.6425
114.3435
66.5075
52.3786
42.4264
39.6059
24.2655
14.6363
14.5447
dr = 94 : 6
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



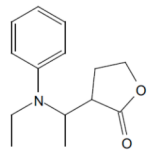
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.30 – 7.21 (m, 3H), 6.85 (dd, *J* = 9.0, 1.1 Hz, 2H), 6.76 (tt, *J* = 7.3, 1.0 Hz, 1H), 4.51 (qd, *J* = 6.9, 3.7 Hz, 1H), 4.35 (td, *J* = 8.8, 2.6 Hz, 1H), 4.13 (ddd, *J* = 9.8, 8.9, 6.9 Hz, 1H), 3.37 – 3.18 (m, 2H), 2.93 (ddd, *J* = 10.8, 9.1, 3.7 Hz, 1H), 2.37 – 2.25 (m, 1H), 2.25 – 2.15 (m, 1H), 1.31 (d, *J* = 6.9 Hz, 3H), 1.15 (t, *J* = 7.0 Hz, 3H).



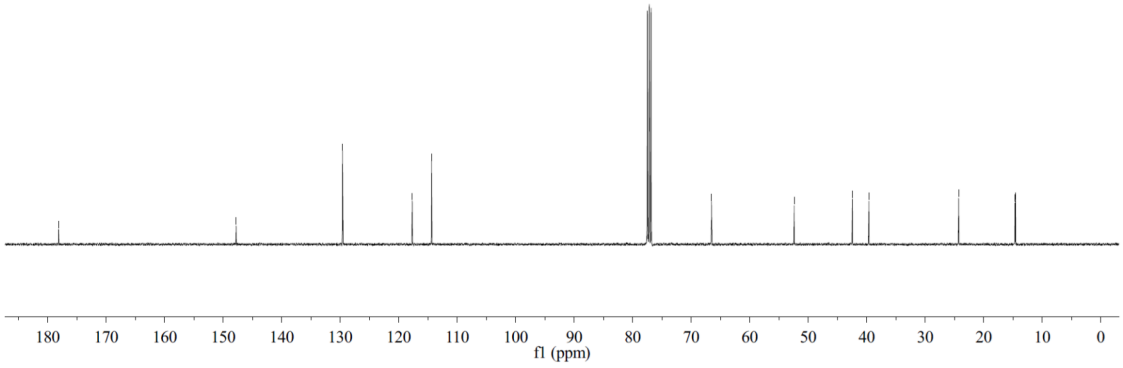
2h-1
major diastereomer with L9

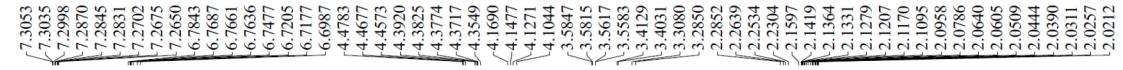


^{13}C NMR (101 MHz, $CDCl_3$) δ 178.12, 147.76, 129.57, 117.65, 114.35, 66.51, 52.38, 42.43, 39.62, 24.27, 14.65, 14.55.

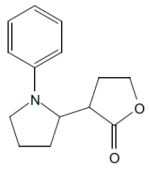


2h-1
major diastereomer with L9

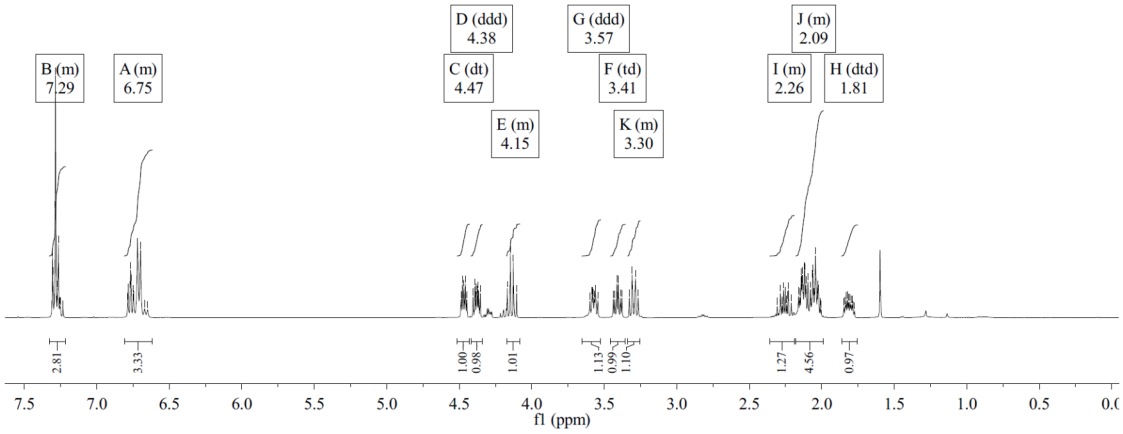




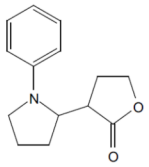
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.33 – 7.22 (m, 2H), 6.81 – 6.62 (m, 3H), 4.47 (dt, *J* = 7.9, 3.7 Hz, 1H), 4.38 (ddd, *J* = 9.0, 6.5, 4.6 Hz, 1H), 4.17 – 4.08 (m, 1H), 3.57 (ddd, *J* = 9.2, 6.9, 5.6 Hz, 1H), 3.41 (td, *J* = 9.9, 3.9 Hz, 1H), 3.34 – 3.25 (m, 1H), 2.36 – 2.19 (m, 1H), 2.18 – 1.99 (m, 4H), 1.81 (dtd, *J* = 12.7, 6.2, 3.5 Hz, 1H).



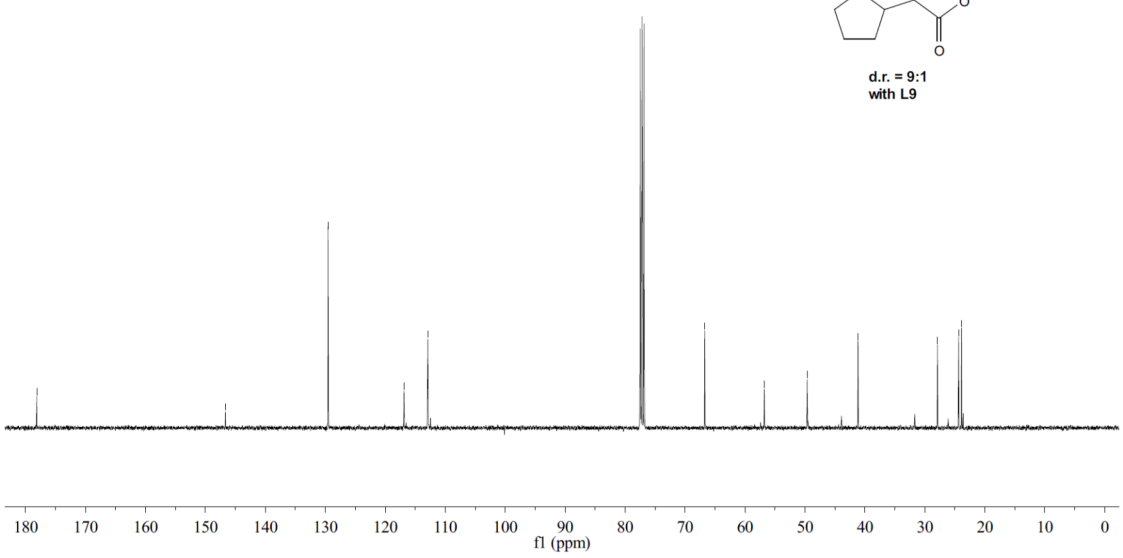
d.r. = 9:1
with L9

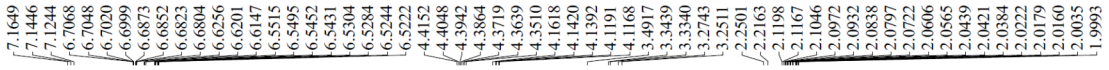


^{13}C NMR (101 MHz, $CDCl_3$) δ 178.13, 146.67, 129.55, 116.89, 112.90, 66.73, 56.78, 49.63, 41.16, 27.89, 24.36, 23.91.

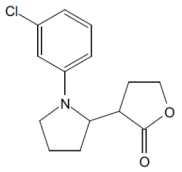


d.r. = 9:1
with L9

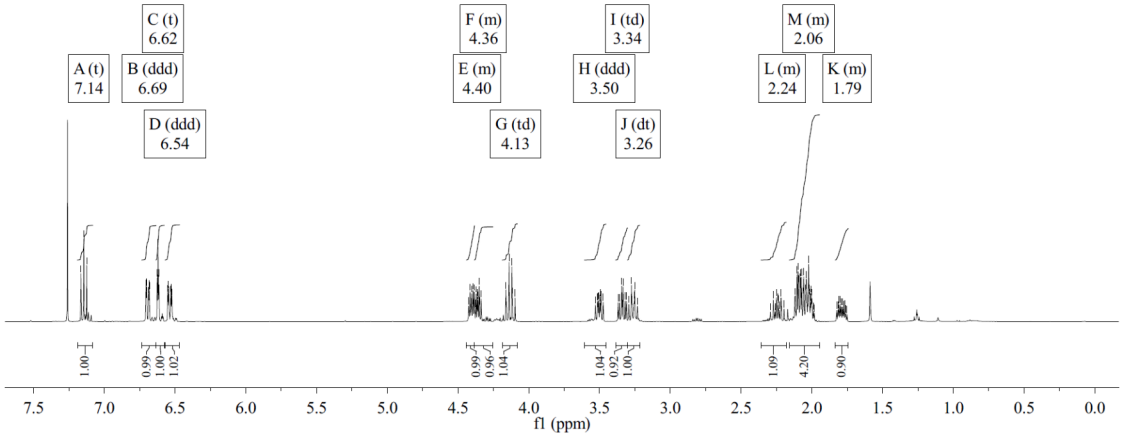




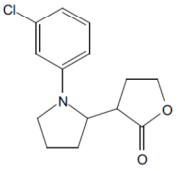
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.14 (t, *J* = 8.1 Hz, 1H), 6.69 (ddd, *J* = 7.8, 1.9, 0.8 Hz, 1H), 6.62 (t, *J* = 2.2 Hz, 1H), 6.54 (ddd, *J* = 8.4, 2.5, 0.8 Hz, 1H), 4.44 – 4.38 (m, 1H), 4.38 – 4.25 (m, 1H), 4.13 (td, *J* = 9.0, 8.0 Hz, 1H), 3.50 (ddd, *J* = 9.3, 7.0, 5.7 Hz, 1H), 3.34 (td, *J* = 10.0, 4.0 Hz, 1H), 3.26 (dt, *J* = 9.3, 7.4 Hz, 1H), 2.36 – 2.18 (m, 1H), 2.16 – 1.95 (m, 4H), 1.83 – 1.74 (m, 1H).



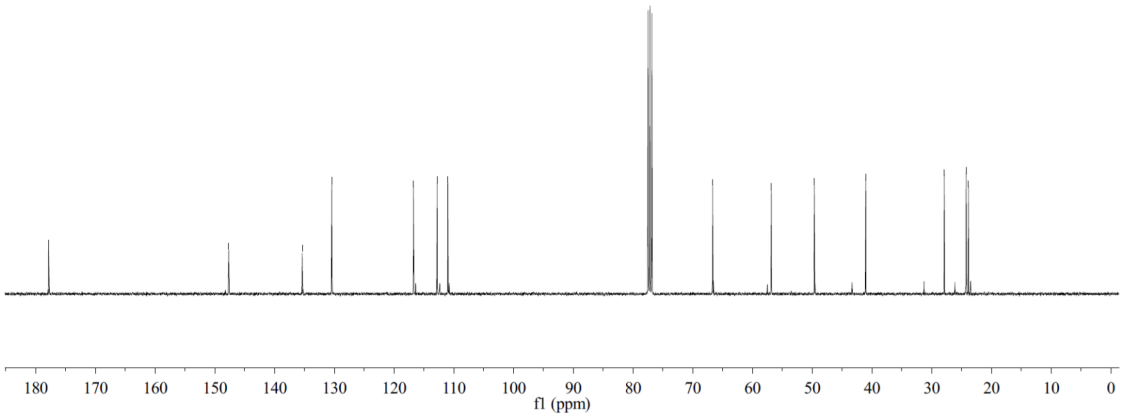
d.r. = 9:1
with L9

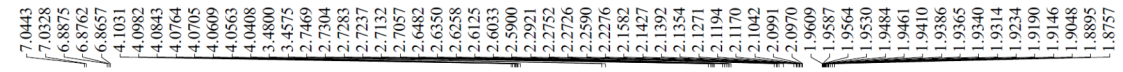


^{13}C NMR (101 MHz, $CDCl_3$) δ 177.81, 147.66, 135.38, 130.42, 116.75, 112.78, 111.00, 66.67, 56.85, 49.65, 41.04, 27.90, 24.25, 23.86.

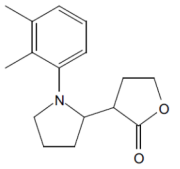


d.r. = 9:1
with L9

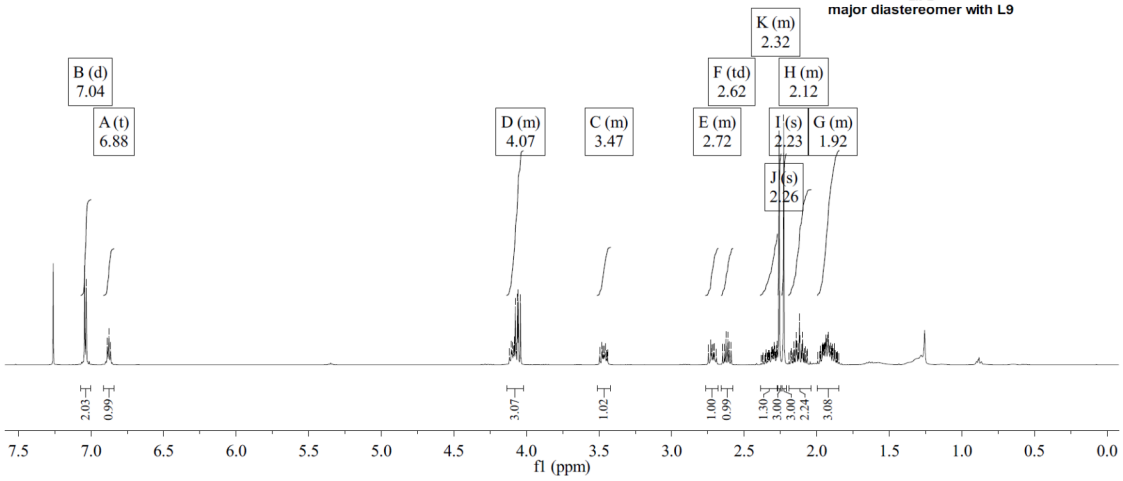




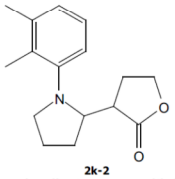
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.04 (d, *J* = 4.6 Hz, 2H), 6.88 (t, *J* = 4.3 Hz, 1H), 4.14 – 4.02 (m, 3H), 3.51 – 3.42 (m, 1H), 2.77 – 2.68 (m, 1H), 2.62 (td, *J* = 9.0, 5.3 Hz, 1H), 2.39 – 2.27 (m, 1H), 2.26 (s, 3H), 2.23 (s, 3H), 2.19 – 2.04 (m, 2H), 2.00 – 1.82 (m, 3H).



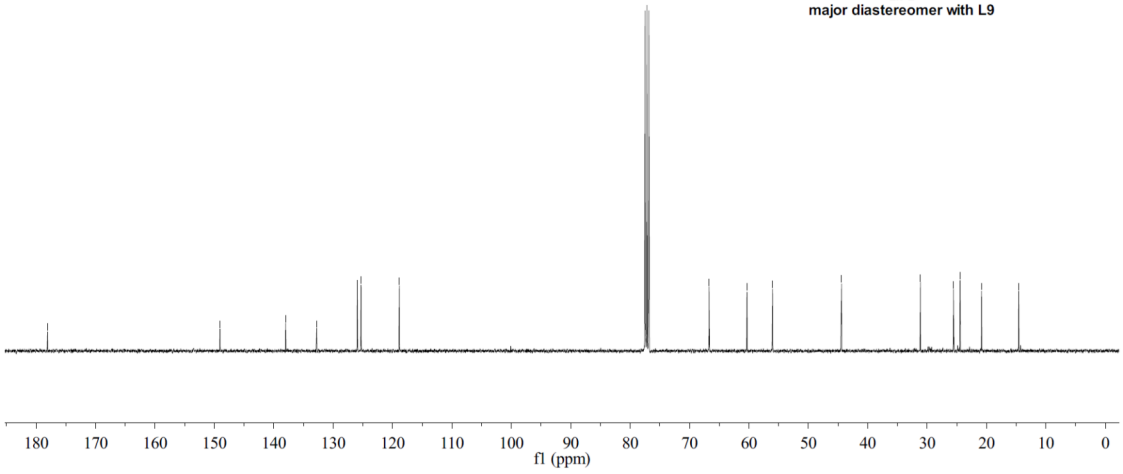
2k-2
major diastereomer with L9

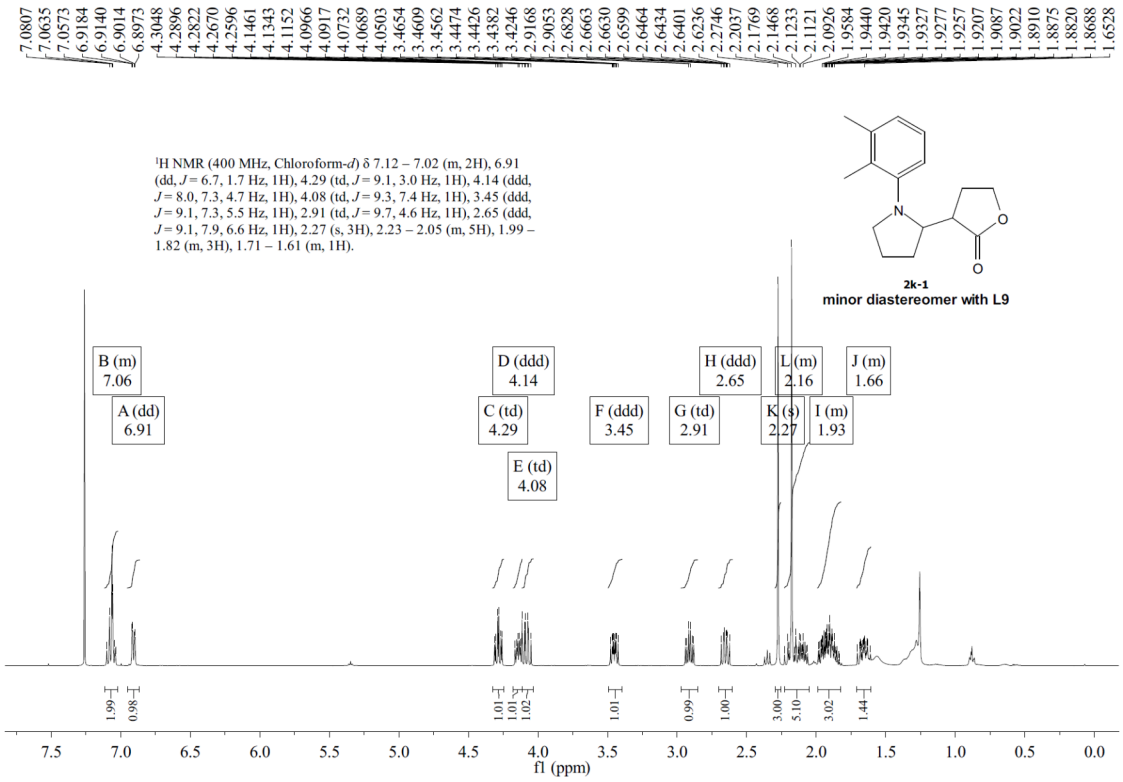


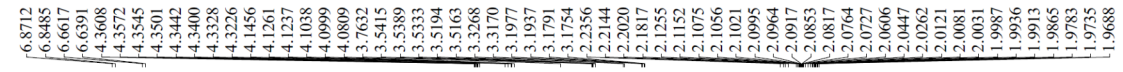
^{13}C NMR (101 MHz, $CDCl_3$) δ 178.08, 149.05, 138.00, 132.78, 125.94, 125.31, 118.85, 66.71, 60.32, 56.02, 44.40, 31.13, 25.56, 24.45, 20.83, 14.59.



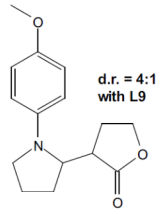
2k-2
major diastereomer with L9



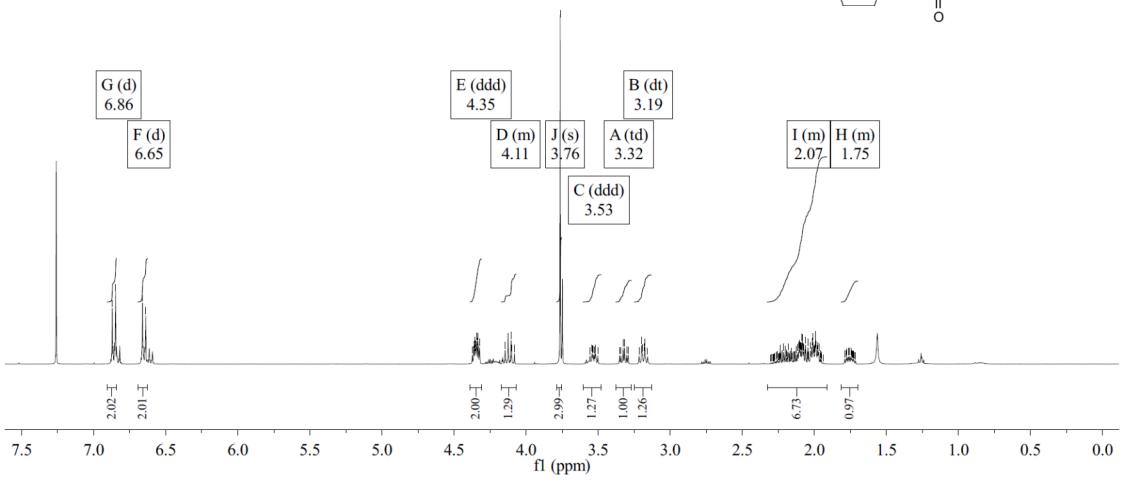

7.0807
7.0635
7.0573
6.9184
6.9140
6.9014
6.8973
4.3048
4.2896
4.2822
4.2670
4.2596
4.1461
4.1343
4.1152
4.0966
4.0917
4.0732
4.0689
4.0503
3.4654
3.4609
3.4562
3.4474
3.4426
3.4382
3.4246
2.9168
2.9053
2.6828
2.6663
2.6630
2.6599
2.6464
2.6434
2.6401
2.6236
2.2746
2.2037
2.1769
2.1468
2.1233
2.1121
2.0926
1.9584
1.9440
1.9420
1.9345
1.9327
1.9277
1.9257
1.9207
1.9087
1.9022
1.8910
1.8875
1.8820
1.8688
1.6528
¹H NMR (400 MHz, Chloroform-d) δ 7.12 – 7.02 (m, 2H), 6.91 (dd, J = 6.7, 1.7 Hz, 1H), 4.29 (td, J = 9.1, 3.0 Hz, 1H), 4.14 (ddd, J = 8.0, 7.3, 4.7 Hz, 1H), 4.08 (td, J = 9.3, 7.4 Hz, 1H), 3.45 (ddd, J = 9.1, 7.3, 5.5 Hz, 1H), 2.91 (td, J = 9.7, 4.6 Hz, 1H), 2.65 (ddd, J = 9.1, 7.9, 6.6 Hz, 1H), 2.27 (s, 3H), 2.23 – 2.05 (m, 5H), 1.99 – 1.82 (m, 3H), 1.71 – 1.61 (m, 1H).
2k-1
minor diastereomer with L9
B (m) 7.06
A (dd) 6.91
D (ddd) 4.14
C (td) 4.29
E (td) 4.08
F (ddd) 3.45
G (td) 2.91
H (ddd) 2.65
L (m) 2.16
K (s) 2.27
I (m) 1.93
J (m) 1.66
1.99
0.98
1.01
1.01
1.02
1.01
0.99
1.00
3.00
5.10
3.02
1.44
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)
178.8757
146.8693
138.2624
132.6887
126.0325
125.2855
117.3672
67.1644
58.5696
54.7760
40.5155
26.1595
23.6116
23.0168
20.7991
14.3603
¹³C NMR (101 MHz, CDCl₃) δ 178.88, 146.87, 138.26, 132.69, 126.03, 125.29, 117.37, 67.16, 58.57, 54.78, 40.52, 26.16, 23.61, 23.02, 20.80, 14.36.
2k-1
minor diastereomer with L9
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



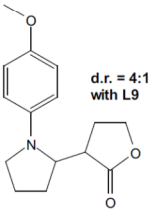
^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.86 (d, *J* = 9.1 Hz, 2H), 6.65 (d, *J* = 9.1 Hz, 2H), 4.35 (ddd, *J* = 8.7, 7.1, 4.4 Hz, 2H), 4.17 – 4.07 (m, 1H), 3.76 (s, 3H), 3.53 (ddd, *J* = 8.9, 6.7, 5.6 Hz, 1H), 3.32 (td, *J* = 9.9, 4.0 Hz, 1H), 3.19 (dt, *J* = 8.8, 7.4 Hz, 1H), 2.33 – 1.91 (m, 5H), 1.81 – 1.70 (m, 1H).



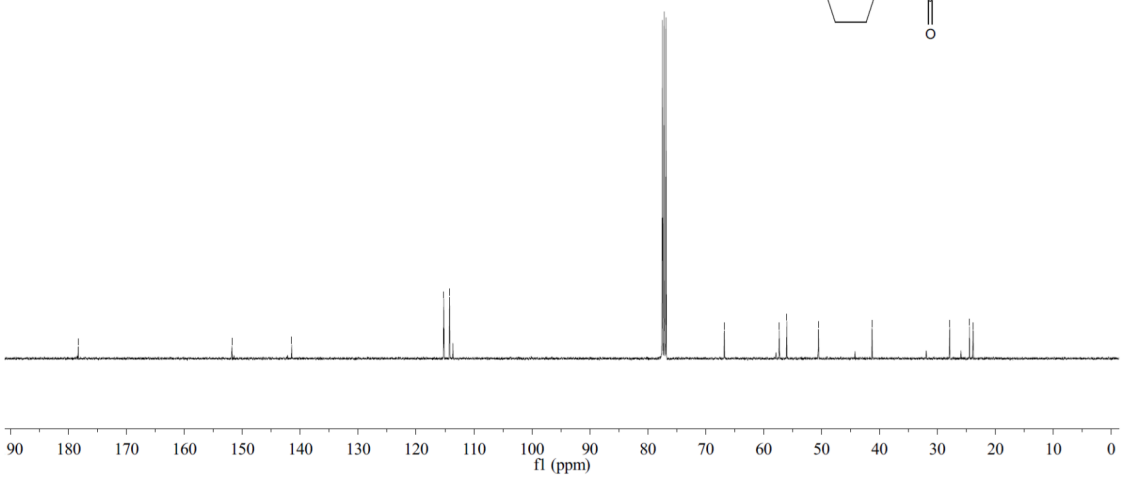
d.r. = 4:1 with L9

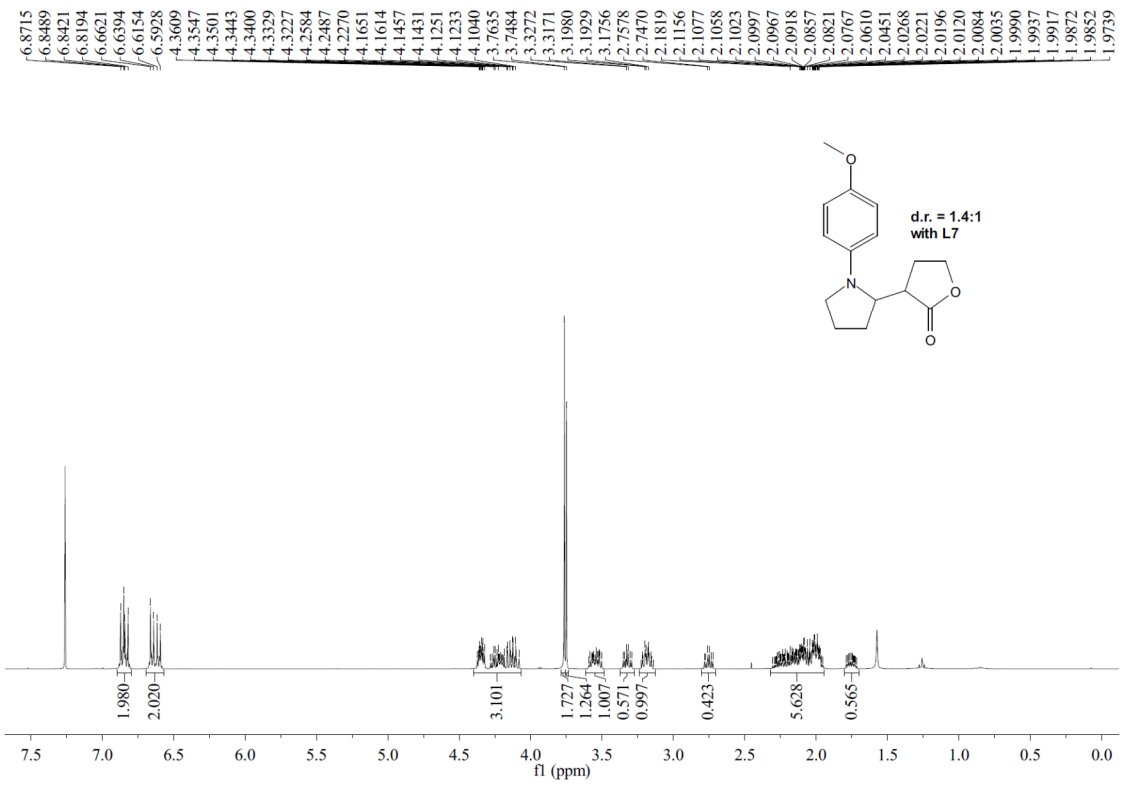


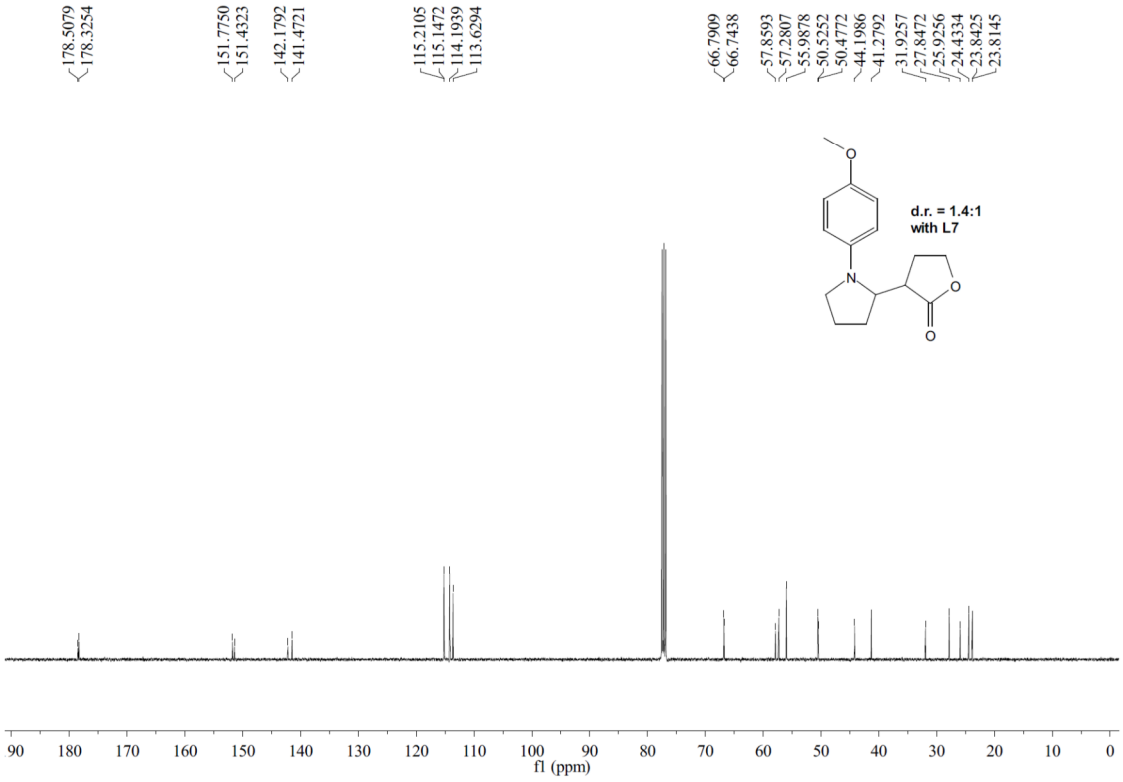
^{13}C NMR (101 MHz, $CDCl_3$) δ 178.33, 151.78, 141.48, 115.21, 114.20, 66.79, 57.28, 55.99, 50.53, 41.28, 27.85, 24.44, 23.82.

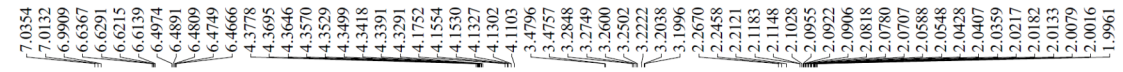


d.r. = 4:1 with L9

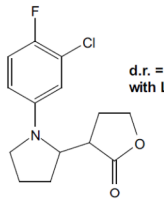



6.8715
6.8489
6.8421
6.8194
6.6621
6.6394
6.6154
6.5928
4.3609
4.3547
4.3501
4.3443
4.3400
4.3329
4.3227
4.2584
4.2487
4.2270
4.1651
4.1614
4.1457
4.1431
4.1251
4.1233
4.1040
3.7635
3.7484
3.3272
3.3171
3.1980
3.1929
3.1756
2.7578
2.7470
2.1819
2.1156
2.1077
2.1058
2.1023
2.0997
2.0967
2.0918
2.0857
2.0821
2.0767
2.0610
2.0451
2.0268
2.0221
2.0196
2.0120
2.0084
2.0035
1.9990
1.9937
1.9917
1.9872
1.9852
1.9739
d.r. = 1.4:1
with L7
1.980
2.020
3.101
1.727
1.264
1.007
0.571
0.997
0.423
5.628
0.565
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

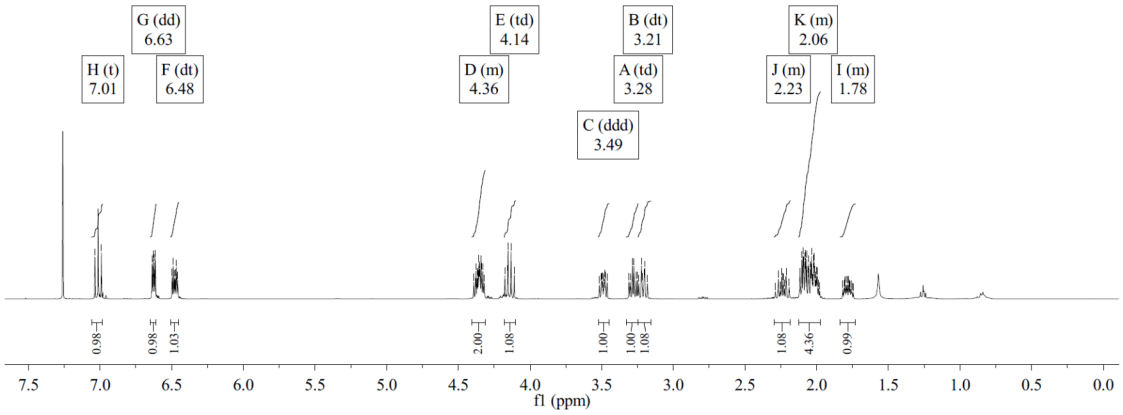

178.5079
178.3254
151.7750
151.4323
142.1792
141.4721
115.2105
115.1472
114.1939
113.6294
66.7909
66.7438
57.8593
57.2807
55.9878
50.5252
50.4772
44.1986
41.2792
31.9257
27.8472
25.9256
24.4334
23.8425
23.8145
d.r. = 1.4:1
with L7
90
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



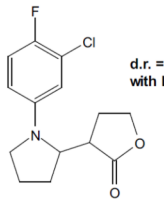
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.01 (t, *J* = 8.9 Hz, 1H), 6.63 (dd, *J* = 6.1, 3.0 Hz, 1H), 6.48 (dt, *J* = 9.1, 3.4 Hz, 1H), 4.40 – 4.31 (m, 2H), 4.14 (td, *J* = 8.9, 7.9 Hz, 1H), 3.49 (ddd, *J* = 9.1, 7.1, 5.5 Hz, 1H), 3.28 (td, *J* = 9.9, 4.0 Hz, 1H), 3.21 (dt, *J* = 9.1, 7.4 Hz, 1H), 2.30 – 2.18 (m, 1H), 2.13 – 1.97 (m, 4H), 1.83 – 1.73 (m, 1H).



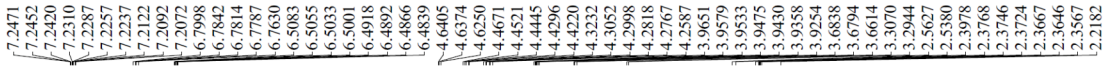
d.r. = 13:1
with L9



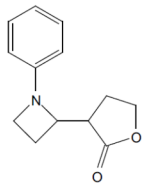
^{13}C NMR (101 MHz, $CDCl_3$) δ 177.80, 150.72 (d, *J* = 239.4 Hz), 143.81 (d, *J* = 2.0 Hz), 121.48 (d, *J* = 18.4 Hz), 117.05 (d, J = 21.8 Hz), 114.02, 111.82 (d, *J* = 6.1 Hz), 66.69, 57.22, 50.19, 41.06, 27.98, 24.36, 23.81.



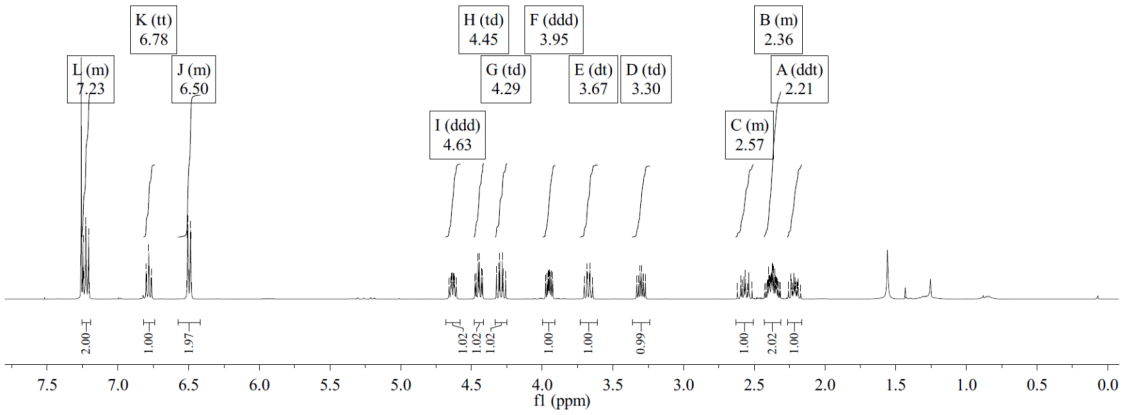
d.r. = 13:1
with L9



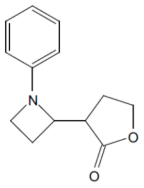
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.25 – 7.19 (m, 2H), 6.78 (tt, *J* = 7.4, 1.1 Hz, 1H), 6.57 – 6.42 (m, 2H), 4.63 (ddd, *J* = 8.3, 7.0, 5.0 Hz, 1H), 4.45 (td, *J* = 9.0, 3.0 Hz, 1H), 4.29 (td, *J* = 9.3, 7.2 Hz, 1H), 3.95 (ddd, *J* = 8.9, 7.1, 4.1 Hz, 1H), 3.67 (dt, *J* = 8.8, 7.3 Hz, 1H), 3.30 (td, *J* = 9.7, 5.1 Hz, 1H), 2.63 – 2.51 (m, 1H), 2.43 – 2.31 (m, 2H), 2.21 (ddt, *J* = 11.4, 8.8, 7.2 Hz, 1H).



single diastereomer with L10



^{13}C NMR (101 MHz, $CDCl_3$) δ 176.93, 151.30, 129.28, 118.40, 111.82, 67.28, 62.19, 50.18, 43.12, 23.63, 18.92.



single diastereomer with L10

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